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VITAMIN D STATUS: ASSOCIATIONS WITH CHRONIC DISEASE RISK FACTORS AND COGNITIVE FUNCTION

A DISSERTATION APPROVED FOR THE
DEPARTMENT OF HEALTH AND EXERCISE SCIENCE

BY

_____
Dr. Michael G. Bemben, Chair

_____
Dr. Debra Bemben

_____
Dr. Allen Knehans

_____
Dr. Mark A. Anderson

_____
Dr. Randa Shehab

I would like to dedicate this to my family: my father Ronald, my mother Christine, and my brother Jeff. They have encouraged me along the way and helped me reach this goal. I could not have done it without all your love and support. Dad, I wish I could have finished earlier so you could have enjoyed this moment with me. I hope I made you proud.

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ABSTRACT

Vitamin D deficiency and/or insufficiency is common in US adults and is linked to increased chronic disease risk. With the identification of vitamin D metabolizing enzymes and the vitamin D receptor in many tissues throughout the body, vitamin D may play a critical role in many bodily processes that effect numerous disease states affecting millions of Americans. Older adults are at increased risk of vitamin D deficiency and/or insufficiency due to decreased cutaneous vitamin D synthesis, low sun exposure, high sunscreen use, and low vitamin D content in the food supply. Identifying a low vitamin D status and correcting it could be an easy initial step for many individuals to improve their overall health and reduce their chronic disease risk. **PURPOSE:** The purpose of the present study was to investigate the relationship between current vitamin D status and risk factors (components of metabolic syndrome and cognitive function) for numerous chronic diseases (cardiovascular disease, diabetes, dementia) in men and women aged 50-70 years. **METHODS:** Seventy-two (72) Caucasian recreationally active older individuals (54 females, 18 males) aged 50-70 years old completed this cross-sectional study. Subjects completed a medical history, food frequency, sun exposure, and international physical activity questionnaire. Subjects had a fasting blood draw taken and lipid panel, glucose, and vitamin D values were measured. The testing visit (~1 hour) included measuring height, weight, waist circumference, peripheral blood pressure, central blood pressure and arterial stiffness (via Pulse Wave Analysis), % body fat (DXA scan), and cognitive function (a specific battery of computerized tests utilizing the Automated Neuropsychological Assessment Metrics (ANAM) test system). Two-way ANOVA (Physical Activity, Gender) was

used to determine group differences for all outcome measures based on physical activity and gender. Pearson correlation coefficients were used to explore potential relationships between serum 25(OH)D levels and disease risk factors. A one-way ANOVA was used to evaluate potential differences across the three levels of vitamin D status (Deficient, Insufficient, Sufficient) for each risk factor for chronic disease. Vitamin D status was also utilized in multiple Chi-Square analyses (gender, physical activity, ANAM scores, vitamin D synthesis (time of year –ability to synthesize vitamin D from the sun or not)). Statistical significance was set at α=0.05. **RESULTS:** Exactly 50% (36 out of 72) of the study population were vitamin D deficient (9) or insufficient (27). Deficient and/or insufficient vitamin D status was associated with GLU, TGs, % BF, and A/G Ratio. When evaluating the correlation between circulating vitamin D levels (25(OH)D) and risk factors, males had a stronger correlation vs. females. For males the correlations were moderate as vitamin D levels were negatively correlated with P_SP ($r = -0.557$; $p=0.016$), P_MEANP ($r = -0.496$; $p=0.036$); C_SP ($r = -0.534$; $p=0.022$). For females, the correlations were significant but weak as vitamin D levels were negatively correlated with GLU ($r = -0.386$; $p=0.004$), TG ($r = -0.296$; $p=0.030$), A/G Ratio ($r = -0.425$; $p=0.001$). No significant main effect for gender for dietary vitamin D ($p=0.171$) or for physical activity for dietary vitamin D ($p=0.105$) was detected. No significant main effect for gender for supplemental vitamin D ($p=0.254$) or for physical activity for supplemental vitamin D ($p=0.695$) was detected. The correlation between dietary vitamin D and circulating levels of vitamin D was low and not significant ($r = 0.171$; $p=0.152$) and gender and physical activity had minimal effect on these relationships. All the correlations between circulating levels of vitamin D and

cognitive test scores were low and not significant. 70% (7 out of 10) of the subjects in the low physical activity group were vitamin D deficient and/or insufficient vs. 51.5% (17 out of 33) and 41.4% (12 out of 29) for the moderate and high physical activity groups respectively. There is a strong association between vitamin D status and the time of year you get your vitamin D levels measured ($p=0.036$). The inability to synthesize vitamin D during the winter months significantly affects vitamin D status, as 62.5% of subjects tested from November to February did not have sufficient vitamin D status. **CONCLUSION:** A high percentage (50%) of this study population was vitamin D deficient and/or insufficient, and it went up to 70% if you were in the low physical activity group. Deficient and/or insufficient vitamin D status is associated with risk factors for chronic disease, as levels for GLU, TGs, % BF, and A/G Ratio all decreased with improved vitamin D status. It is more difficult to maintain a sufficient vitamin D status (> 30ng/ml) during the winter months when most people in the US cannot synthesize vitamin D from the sun. Individuals need to pay attention and be more diligent with their dietary and possibly supplemental vitamin D intake, especially during the winter months, in order to maintain a sufficient vitamin D status and take advantage of all the potential benefits vitamin D has to offer for overall health and reduced chronic disease risk.

CHAPTER I

INTRODUCTION

There is a renewed interest in vitamin D research, as vitamin D publications have notably increased over the last decade. A new Institute of Medicine (IOM) report on vitamin D (and calcium) changed the vitamin D recommendations, which vitamin D researchers largely disagreed with and as a result have published over 12,000 vitamin D articles since the IOM report in 2011 (46, 102, 110). There is good reason for this renewed interest, as vitamin D deficiency is common in US adults, especially among minority groups (36, 44, 53, 54, 65, 117), and with recent discoveries of critical roles played by vitamin D in many other bodily processes besides calcium and bone metabolism (58, 59, 61), correcting deficiencies/insufficiencies could have many beneficial effects for numerous disease states affecting millions of Americans. Recent discoveries of a vitamin D receptor (VDR) in multiple cell types as well as the presence of the activating vitamin D enzyme (1α-hydroxylase) in many tissues (3, 28, 29, 72) has led to an appreciable spike in the research of possible non-skeletal benefits of vitamin D, although some controversy exists over which tissues actually express the VDR due to the amount of the receptor present and the quality of the antibodies utilized (124, 126).

Recent Institute of Medicine (IOM) recommendations (102) for vitamin D (600 IU/day for 1-70 yrs. old, 800 IU/day for >70 yrs. old), according to prominent expert vitamin D researchers, seem to have been created with only skeletal health benefits in mind, and even skeletal needs require higher recommendations according to these experts. Interestingly, due to perceived skin cancer risk and variability in cutaneous

vitamin D synthesis via ultraviolet (UV) light, these new Recommended Dietary Allowances (RDAs) for vitamin D were created for an individual receiving minimal sun exposure. Non-skeletal benefits of vitamin D were deemed inconclusive in the IOM report due to insufficient evidence in their opinion, yet their new recommendations appear insufficient and inconsistent with results seen by leading vitamin D researchers(57). Complicating this issue even further is the fact that overweight or obese individuals (~2/3 of the population) have a more difficult time raising their vitamin D status with intake at the new Dietary Reference Intake (DRI) levels(41, 57, 77, 132). This inconsistency with modern research results and seemingly blatant disregard for the status and size of the US population seems to undermine the credibility of the IOM report for these new vitamin D recommendations.

Vitamin D's history is linked with the childhood bone disease rickets, with vitamin D deficiency being a by-product of the Industrial Revolution. This industrialization of cities led to many new tall buildings, increased pollution, and increased indoor activity, all of which decreased exposure to sunshine and the optimal endogenous production of vitamin D. It was suspected by numerous doctors that exposure to sunlight had something to do with bone development, but this theory was panned by the scientific community, as they could not accept that simply exposure to sunlight could cure this bone-deforming disease. Decades passed with minimal progress in curing rickets for the rest of the 19th century, and by the dawn of the 20th century, rickets was rampant in industrialized cities across Europe and the US. Early in the 20th century, the link between sunlight and skeletal health was more widely accepted, with vitamin D being discovered in the 1920's by scientists such as Mellanby,

McCollum, Huldshinsky, and Steenbock and the structure of vitamin D uncovered soon after in the early 1930's(29, 55). Unfortunately, rickets is not a disease that disappeared with the discovery of vitamin D, as lately cases are on the rise again. This is due to a number of reasons, including low vitamin D content in human breast milk, children spending too much time indoors, increased sunscreen use, and increased use of protective clothing when outdoors(29, 65).

The major source of vitamin D in humans is exposure of the skin to sunlight, specifically ultraviolet B (UVB) radiation that is between 290-315 nm. These UVB photons are absorbed by 7-dehydrocholesterol (7-DHC) in the skin and quickly form previtamin D_3. This unstable previtamin D_3 thermally isomerizes to vitamin D_3 which can then bind to the vitamin D binding protein (DBP) in the circulation as it travels to the liver. In the liver it is converted to 25-hydroxyvitamin D (25(OH)D), which is the major circulating form of vitamin D and is also what is measured to determine an individuals' vitamin D status. Vitamin D is unique in that the major circulating form (25(OH)D) that is measured to determine vitamin D status is not actually the active form. 25(OH)D has to travel to the kidneys where it is converted to 1,25-dihydroxyvitamin D ($1,25(OH)_2D$). This active form ($1,25(OH)_2D$) then enters the circulation to travel to target tissues that regulate calcium metabolism, such as the intestine (66). $1,25(OH)_2D$ interacts with a vitamin D receptor (VDR) in the intestine to increase epithelial calcium channels to enhance calcium transport into the absorptive cell. $1,25(OH)_2D$ also helps calcium transport across the absorptive cell into the circulation via increased expression of a calcium binding protein (calbindin9k) (26, 27). Since vitamin D is necessary for proper absorption of calcium in the small intestine, it

helps ensure adequate calcium deposition to the bones to give them their strength. In addition to the importance of maximizing your peak bone density in your adolescence and early twenties, vitamin D continues to play a crucial role in bone health throughout later life. Bone is very metabolically active, continually being broken down and rebuilt (bone remodeling) throughout your life. Unfortunately, starting in your late 30's you begin to break down more bone than is rebuilt, thus throwing off this remodeling process slightly in favor of a gradual loss of bone density and increased fragility as you age. Adequate vitamin D intake and status will help maximize calcium absorption, leading to normal bone mineralization, and will help minimize and slow this improper remodeling as you age.

So why is our vitamin D status poor and getting worse? People get a very high percentage (90-95%) of their vitamin D requirement from exposure to sunlight, specifically exposure to ultraviolet B (UVB) radiation. Unfortunately, most adults in the US do not go outside in the sun enough to meet their vitamin D needs year round. Also, during the winter months, it is not possible in much of the US (above 33° north latitude (Atlanta, GA)) (67, 127, 129) for cutaneous vitamin D production. Combine this with the added obstacles for individuals with darker skin (due to increased melanin content, which decreases vitamin D synthesis) and older individuals (less 7-DHC content in skin with aging) and you start to understand why many adults, especially older adults are vitamin D insufficient and deficient (47, 51). UVB is the only form of UV radiation that is absorbed by the vitamin D precursor in the skin to make vitamin D. The mass media has increased the coverage of the potential benefits of vitamin D, leading to more patients asking their doctors about their vitamin D status, and the

25(OH)D assay is now one of the most ordered assays in the US (65). But with the previously mentioned increase in sunscreen use and less time spent outdoors, combined with the fact that vitamin D is not widely available in the food supply, many individuals are at risk for vitamin D deficiency.

It is noteworthy to highlight the challenges that go into accurately assessing vitamin D status. It is important to use a combination of a blood 25(OH)D measurement along with questions regarding one's dietary (food and supplemental) vitamin D intake and sunlight exposure (87). Sunlight questionnaires need to be comprehensive enough so that they take into account all the factors that affect one's personal UVB exposure and vitamin D synthesis via skin. These factors include latitude, season, time of day, sunscreen use, age, clothing, and skin type (30, 35, 85, 127, 128). Vitamin D status is determined by measuring 25(OH)D levels in the blood, which is the major circulating form of vitamin D. 25(OH)D includes vitamin D intake from food and vitamin D from UVB exposure, and has a half-life of ~2-3 weeks (64). While 25(OH)D is not the biologically active form ($1,25(OH)_2D$), its circulating levels are 1000 times more than $1,25(OH)_2D$. Combine that with the fact that $1,25(OH)_2D$ has a half-life of only 4-6 hours, and it is clear that measuring 25(OH)D is the best option (59, 62, 64).

Most experts agree that vitamin D deficiency be defined as having a 25(OH)D level of <20 ng/ml, and vitamin D insufficiency is between 21-29 ng/ml. Ideally, a level of >30 ng/ml is preferred by most experts (64, 65). This level of >30 ng/ml is very difficult to reach if you don't have consistent sensible sun exposure, but the public is

being frightened over the past few decades by the pharmaceutical industry and dermatologists to avoid the sun at all times. These industries have deep pockets to get their message out to the public that "no amount of unprotected sun exposure is sensible or important for health" (65), and to use their sunscreen products constantly in this youth obsessed society. A balance needs to be struck though, as it is important to point out that you can take advantage of all the benefits of sensible sun exposure while minimizing its aging effects or skin cancer risk(88). The research to be covered in the next chapter will illustrate that sensible sun exposure and adequate vitamin D status appear to reduce many chronic disease risk factors and risk for cognitive dysfunction (memory, attention, processing speed), and this benefit far outweighs any skin cancer risk or premature aging from exposure to UVB radiation.

Purpose

The purpose of the present study was to investigate the relationship between current vitamin D status and risk factors (components of metabolic syndrome and cognitive dysfunction) for numerous chronic diseases (cardiovascular disease, diabetes, dementia) in men and women aged 50-70 years. This study aimed to determine if deficient and/or insufficient vitamin D status is associated with risk factors (components of metabolic syndrome and cognitive dysfunction) for chronic disease (cardiovascular disease, diabetes, dementia).

Research Questions

1. What is the prevalence of deficient and/or insufficient vitamin D status in a sample of older subjects?
2. Is a deficient and/or insufficient vitamin D status associated with risk factors (components of metabolic syndrome and cognitive dysfunction) for chronic disease (cardiovascular disease, diabetes, dementia)?
3. Does circulating vitamin D levels (25(OH)D) affect risk for chronic disease and are these relationships different for males and females?
4. Does dietary intake of vitamin D differ based on gender or physical activity?
5. Does supplemental intake of vitamin D differ based on gender or physical activity?
6. Is the relationship between dietary intake of vitamin D and circulating levels of vitamin D (25(OH)D) different based on gender or physical activity?
7. Does circulating vitamin D levels (25(OH)D) correlate with Throughput scores on cognitive tests?

Research Subquestions

1. A) What is the prevalence of deficient and/or insufficient vitamin D status based on gender?

   B) What is the prevalence of deficient and/or insufficient vitamin D status based on physical activity level?

2. A) Is a deficient and/or insufficient vitamin D status associated with risk factors (components of metabolic syndrome and cognitive dysfunction) for chronic disease (cardiovascular disease, diabetes, dementia) differently based on gender? B) Is a deficient and/or insufficient vitamin D status associated with risk factors (components of metabolic syndrome and cognitive dysfunction) for chronic disease (cardiovascular disease, diabetes, dementia) differently based on physical activity levels?

Research Hypotheses

1. The vitamin D status of at least 50% of these older individuals will be deficient and/or insufficient.
2. Deficient and/or insufficient vitamin D status will be associated with chronic disease risk factors.
3. Circulating vitamin D levels (25(OH)D) will correlate with risk factors for chronic disease and males will have stronger correlations with risk factors for chronic disease vs. females.
4. Dietary intake of vitamin D will not differ based on gender or physical activity level.
5. Supplemental intake of vitamin D will not differ based on gender or physical activity level.
6. Correlations between dietary intake of vitamin D and circulating levels of vitamin D (25(OH)D) will not differ based on gender or physical activity level.

7. Circulating vitamin D levels (25(OH)D) will not correlate with Throughput scores on cognitive tests.

Research Subquestion Hypotheses

1. A) The prevalence of deficient and/or insufficient vitamin D status will be greater in males vs. females.

   B) The prevalence of deficient and/or insufficient vitamin D status will be greater in the low physical activity group vs moderate or high physical activity group.
2. A) Males with a deficient and/or insufficient vitamin D status will be associated with more risk factors for chronic disease vs. females with a deficient and/or insufficient vitamin D status.

   B) Subjects in the low physical activity group with a deficient and/or insufficient vitamin D status will not see a significant difference in risk factors for chronic disease (across levels of vitamin D status) vs subjects in the moderate or high physical activity groups with a deficient and/or insufficient vitamin D status.

Significance of the Study

There appears to be a renewed interest in researching vitamin D, as annual citations in the PubMed database on vitamin D have doubled in the past decade (2). Also in the first decade of the 21st century the amount of money spent on vitamin D supplements has increased tenfold from ~$40 million/yr. to over $400 million/yr. (84). The reason for this is that vitamin D deficiency is common in the US adult population,

and with its deficiency linked to increased chronic disease risk and all-cause mortality (36, 76), testing for vitamin D deficiency may need to be part of future chronic disease risk screening procedures. Vitamin D deficiency is common due to the fact that it is not widely available in the food supply and thus very difficult to meet needs through diet alone. Combine this with the fact that the public has been bombarded by the antisun campaigns (pharmaceutical companies and dermatology profession) that any sun exposure should be avoided or only done with their sunscreen products used, it becomes very difficult to maintain adequate vitamin D status. Even the government backed Healthy People 2010 stated an objective that included protective measures to avoid sun exposure during peak hours (10am-4pm) and utilize sunscreen and protective clothing when exposed to sun (85). In fact, a SPF 15 decreases your ability to make vitamin D by about 95%, and an SPF 30 reduces your ability by 99% (65). With everybody constantly using SPF products and with US society experiencing a worsening obesity epidemic, which is linked to vitamin D deficiency/insufficiency (2), now is the time to look at vitamin D's link to chronic disease risk and hopefully checking vitamin D status will become commonplace in attempting to halt chronic disease progression.

Assumptions

1. Subjects answered the questionnaire addressing their nutrition history, sunlight exposure and their medications use honestly and accurately.
2. DXA was a valid and reliable method for measuring % body fat and A/G Ratio.

Delimitations

1. Men and women aged 50-70 years old.
2. Participants were recruited from Norman, OK (USA) and surrounding communities.
3. DXA (Lunar Prodigy, Madison) was used for measuring % body fat and android/gynoid (A/G) ratio.

Limitations

1. Since data was collected in Norman, OK, the results may not be applicable for older individuals with different ethnicities/composition compared to the population of Norman, OK.
2. All subjects participating in the study were volunteers and therefore may not accurately represent the population of older individuals.
3. This study is a cross-sectional design, thus no cause and effect relationship can be implied.

Inclusion Criteria

1. Men and women aged 50-70 years old.
2. Subjects weighing no more than 300 lbs. which is the weight limit of the DXA machine.
3. Subjects should have no cognitive problems that can interfere with their participation. Subjects must score greater than or equal to 27 points (out of 30) on the Mini Mental State Examination (MMSE) to indicate a normal cognition.

4. Subjects should be able to speak and understand English.

Exclusion Criteria

1. Individuals <50 years old or > 70 years old.
2. Subjects weighing more than 300 lbs.
3. Subjects with cognitive impairment that does not allow them to complete the current process or testing. Subjects scoring less than 27 points (out of 30) on the MMSE.

Operational Definitions

1. Vitamin D status – Refers to levels of 25-hydroxyvitamin D, the major circulating form of vitamin D in the blood. The unit of measurement used for vitamin D is nanograms per milliliter (ng/ml). 2.5 nmol/L = 1 ng/ml
2. Rickets – A vitamin D deficiency disease seen in children, which leads to a softening and weakening of the bones.
3. Osteoporosis - A disease of the skeleton characterized by low bone mineral density and micro-architectural deterioration, leading to bone fragility and increased risk of fracture.
4. Ultraviolet B Radiation (UVB) – invisible radiation that comes from the sun with wavelengths of 290-315 nm.
5. Metabolic Syndrome – A name for a group of risk factors that occur together and increase the risk for cardiovascular disease, stroke, and type II diabetes.

According to the American Heart Association, metabolic syndrome is present if someone has any 3 of the following symptoms:

- Blood pressure equal to or higher than 130/85 mmHg
- Fasting blood sugar (glucose) equal to or higher than 100 mg/dL
- Large waist circumference (length around the waist):
  - Men – 40 inches or more
  - Women – 35 inches or more
- Low HDL cholesterol:
  - Men – under 40 mg/dL
  - Women – under 50 mg/dL
- Triglycerides equal to or higher than 150 mg/dL

6. Alzheimer's Disease (AD) – The most common form of dementia, AD is a progressive disorder that affects memory, thinking, and behavior.
7. Dementia – A more general term that refers to a loss of brain function that occurs with certain diseases. It affects memory, thinking, language, judgment, and behavior.
8. Vascular Dementia – The 2nd most common form of dementia. High blood pressure, high cholesterol, atherosclerosis, and diabetes are all risk factors for vascular dementia.
9. Hypovitaminosis D – A 25(OH)D level of less than 30 ng/ml.
10. Dual Energy X-Ray absorptiometry (DXA): Body composition modality that uses two contrasting x-ray beams to yield total and regional lean body mass.

DXA calculates the attenuation values of photons that pass from the x-ray tube through the measurement site of interest.

CHAPTER II

REVIEW OF LITERATURE

Introduction

The role of vitamin D in calcium metabolism and bone health is well documented, and while this remains front and center in its function, new biological processes have recently been identified for vitamin D that expands its impact and calls for a reevaluation of the current intake recommendations. With the discovery of vitamin D receptors and 1α-hydroxylase enzyme in multiple tissues throughout the body, these tissues can convert 25(OH)D to $1,25(OH)_2D$ locally, exerting an autocrine effect that can influence the expression of hundreds of genes in these tissues (97, 115). Holick (60, 61) suggests that these tissues and cells will be able to produce enough $1,25(OH)_2D$ for local biological functions only if circulating levels of 25(OH)D are above 30 ng/ml. This is very difficult to attain through dietary means, as there are few foods that naturally contain vitamin D. Fatty fish (salmon, mackerel), egg yolks, and cod liver oil are a few examples, and fortified foods (milk, yogurt, orange juice, grains) offer 100 IU per serving. Thus, if you are someone that does not eat fish regularly or skips breakfast, which millions of Americans do, then your dietary vitamin D intake will be very low. For every 100 IU of vitamin D ingested daily, the blood level of 25(OH)D increases by 1 ng/ml (56). Thus, many experts recommend 1000-2000 IU of vitamin D/day to maintain vitamin D sufficiency and maximize the potential benefits through local production and autocrine action (62). This local production of $1,25(OH)_2D$ never enters the circulation to effect calcium metabolism, as this could be dangerous. Instead it is self-regulated through the activity of 24-hydroxylase to create

24,25$(OH)_2$D, which was discovered shortly after the discovery of vitamin D. This 24-hydroxylation breaks down 1,25$(OH)_2$D to calcitroic acid, which is biologically inactive and is excreted in the bile (13, 63, 68, 92). Thus after performing its local duties, 1,25$(OH)_2$D self-destructs and the process repeats itself as needed as long as substrate supplies (25(OH)D) are sufficient.

With these recent discoveries of 1,25$(OH)_2$D production in many tissues throughout the body, it then becomes paramount to optimize your substrate (25$(OH)_2$D) levels to reap the potential benefits. Unfortunately, overweight and obesity have an inverse relationship with 25 (OH)D levels (19, 70, 74, 90, 100, 132), and with ~2/3 of the population either overweight or obese, this appears to be one more hurdle for the population to attain sufficient vitamin D status. Combine this with the low sun exposure, high sunscreen use, and low vitamin D content in the food supply, and it becomes clear why no one is immune to vitamin D deficiency and why high rates of vitamin D deficiency have been seen in the US and throughout the world (36, 44, 59, 60, 62).

The purpose of the present study was to investigate the relationship between current vitamin D status and risk factors (components of metabolic syndrome and cognitive dysfunction) for numerous chronic diseases (cardiovascular disease, diabetes, dementia) in men and women aged 50-70 years. This study aimed to determine if deficient and/or insufficient vitamin D status is associated with an increased number of risk factors (components of metabolic syndrome and cognitive dysfunction) for chronic disease (cardiovascular disease, diabetes, dementia). The sections of this chapter are

presented in the following order: 1) Vitamin D activation and metabolism, 2) Vitamin D and overweight/obesity, 3) Vitamin D and type 2 diabetes, 4) Vitamin D and cardiovascular disease, 5) Vitamin D and cognitive function, and 6) Summary.

Vitamin D Activation and Metabolism

With vitamin D available from a scarce number of natural food sources, production of vitamin D via the skin has been the most important source of vitamin D for the vast majority of individuals. UVB radiation is absorbed by 7-dehydrocholesterol (7-DHC; provitamin D_3), a cholesterol precursor located within the triglycerides in the plasma membranes of skin cells in the epidermis. This causes the photolysis of 7-DHC, which causes a conformational change and previtamin D_3 is formed in the keratinocytes of the epidermis. This is a tightly regulated process so that humans cannot experience vitamin D intoxication due to excess sun exposure. With continued sun exposure, only ~15% of 7-DHC is converted to and remains previtamin D_3, and the rest is converted into biologically inactive metabolites (lumisterol, tachysterol). A similar situation occurs at the next step with the cutaneously synthesized vitamin D_3, as continued excess sun exposure will induce the vitamin D_3 to absorb the UVB radiation and be converted and photodegraded to suprasterols and metabolites with no calcemic activity. These photoproducts do have potential positive biologic effects and are being investigated for possible anti-tumor and antiproliferative activity in skin cells, thus sensible sun exposure results in cutaneously produced vitamin D_3 and photoproducts that may offer additional benefits over simple dietary and/or supplemental vitamin D_3. Under a more sensible, non-excessive sun exposure scenario, the formed previtamin D_3 is unstable

and is fairly rapidly (~50% within 2 hours) converted to vitamin D_3, leaves the plasma membrane, and diffuses into the dermal capillary network where it binds to vitamin D binding protein (DBP) and is transported to the liver (116, 120). Vitamin D binding protein (Gc-globulin) is a multifunctional plasma protein that is synthesized and secreted by hepatocytes, and is the major transporter of vitamin D and its metabolites in the circulation. Vitamin D binding protein is a highly polymorphic protein, and the effect of DBP polymorphism on circulating 25(OH)D levels has been studied, as well as the response to oral vitamin D supplementation (17, 24, 38, 123). When vitamin D deficiency is present, it is currently not known the exact role the DBP polymorphism plays. Does it simply involve a difference in binding capacity or are there problems during multiple points along the vitamin D endocrine system. The precise role of DBP when 25(OH)D is delivered to extra renal tissues is still unclear, as there is a "free hormone" hypothesis that states that 25(OH)D not bound to DBP (~1%) is more bioavailable and free to cross the cell membrane. If a person or ethnic group has a DBP genotype that exhibits a lower binding capacity, how does this affect the amount of $1,25(OH)_2D$ created and the influence it eventually could have on a large number of biologic pathways that affect chronic disease risk (130). Vitamin D_3 created in the skin is metabolically inactive and must still be converted to its final active hormone form via a two-step hydroxylation. The first hydroxylation step occurs in the liver and involves a 25-hydroxylation (via 25-hydroxylase) to produce 25(OH)D, the major circulating form found in the blood and the form used to determine vitamin D status. The second hydroxylation step is catalyzed by 1α-hydroxylase to create the active form, $1,25(OH)_2D$. With 25(OH)D and $1,25(OH)_2D$ being discovered over 40 years ago, the

search for the enzymes responsible for vitamin D activation was the next challenge. The enzyme CYP27B1 was confirmed in 1997 as the sole 1α-hydroxylase in all tissues for the creation of the active form (37, 89, 107, 111, 113). On the other hand, the search for the specific enzyme catalyzing the 25-hydroxylation step has proven much more difficult. Recent evidence points to the enzyme CYP2R1 as being of major importance in the bioactivation of vitamin D_3 in humans, but it is too early to completely discount other 25-hydroxylases, including any that may still be discovered (135).

The end result is the active form of vitamin D, $1,25(OH)_2D$, which binds to the vitamin D receptor (VDR), a transcriptional factor, to regulate gene expression. The VDR is a member of the nuclear receptor superfamily acting as a ligand-induced (via $1,25(OH)_2D$) transcription factor. This complex combines with the retinoid X receptor (RXR) to form a heterodimer, which then binds to specific vitamin D response elements (VDREs) to initiate transcription in the regulatory region of vitamin D target genes. Until recently it was thought that the VDR is expressed in most cells of the body, but with advancing scientific techniques, the identification of a highly specific VDR antibody puts this train of thought in serious doubt. With the majority, if not all vitamin D functions being mediated by the VDR, accurately identifying VDRs in tissues throughout the body is critical to understanding the biological significance of vitamin D and uncovering potential novel therapies involving the VDR (124, 126).

The VDR is found in a number of tissues besides those associated with classic vitamin D functions (kidney, bone, intestine, parathyroid gland). These numerous non-classic potential targets for $1,25(OH)_2D$ are currently evolving and under investigation,

but evidence seems to indicate a role for vitamin D beyond its role in regulating calcium homeostasis and bone formation. Caution should be taken though as clarification of VDR expression in tissues such as brain, heart, muscle, and liver needs to occur with specific and sensitive VDR antibody along with a proper negative control. Many previous antibodies used to identify VDR in various target tissues have been found to interact nonspecifically with unknown proteins which could explain a potential false positive for VDR expression. Thus Wang et al recently identified an antibody (D-6) that is highly specific, sensitive, and versatile for identifying VDR in target tissues utilizing numerous immunological methods (124). There are still a number of questions that need to be answered regarding VDR expression. How much VDR expression in a tissue is needed or is optimal for function? What is the function of VDR in certain tissues? (There are high levels of VDR expression in tissues where the function has yet to be definitively determined (pancreas, keratinocytes)) What role do factors such as vitamin D status, age, and health have on VDR expression in tissues? Does isolating cells from tissues or using cultured cells affect VDR expression and thus gene transcription? Answering these questions will go a long way to better understanding the function of vitamin D in the cells and tissues where VDR expression is definitely found. As we progress through the following sections, VDR expression in the cells/tissues involved with the chronic disease risk factors will be discussed. When we are discussing VDR expression in relation to chronic disease risk factors, researchers are essentially investigating VDR playing a protective role against these conditions. With research ongoing at all these possible sites of VDR expression, there is also evidence of extra renal expression of CYP27B1 so that numerous tissues can synthesize

$1,25(OH)_2D$ and are capable of autocrine/paracrine functions. With this capability, it is also important that these tissues have the ability to degrade 25(OH)D and $1,25(OH)_2D$ so that excess amounts do not get into the bloodstream, influence calcium metabolism and lead to hypercalcemia. This inactivation of active vitamin D is accomplished by the catabolic mitochondrial 24-hydroxylase enzyme CYP24A1, which is transcriptionally induced by $1,25(OH)_2D$ in these extrarenal tissues. With CYP27B1 enabling cellular concentrations of $1,25(OH)_2D$ to rise, CYP24A1 works in conjunction with CYP27B1 to balance and refine tissue exposure to the active vitamin D hormone. Target cell modification is crucial, as CYP24A1 fulfills the role of degrading $1,25(OH)_2D$ after appropriate alterations in gene expression have occurred (71, 104).

Vitamin D and Overweight/Obesity

It has been consistently reported in the literature that obese individuals are shown to have lower 25(OH)D levels compared to non-obese individuals, and further investigation into possible mechanisms is warranted as it has not been completely described to this point. With the percentage of overweight/obese individuals in the US approaching 70% of the population and no slowdown in sight, important questions need to be addressed. Is there an altered vitamin D metabolism in overweight/obese populations? What effect does vitamin D supplementation have on this portion of society vs. healthy weight individuals? Do overweight/obese individuals need a higher vitamin D supplementation level to correct a deficiency? Is the response to vitamin D supplementation dependent on body size? How does vitamin D status affect obesity? What are the effects of weight loss on 25(OH)D levels and vitamin D status? What

direction must future research take to uncover the role of vitamin D supplementation in obesity prevention (118)?

It has been reported by numerous researchers that overweight/obese individuals tend to have a worse vitamin D status than those with less adipose tissue (19, 70, 74, 90, 100, 132). Wortsman et al. (132) found that obese (BMI>30) subjects had lower basal 25(OH)D concentrations than age-matched control subjects (BMI<25) (20.0 ± 3.4 ng/ml vs 33.9 ± 4.1 ng/ml); ($p=0.017$). In the same study, after an identical exposure to UVB irradiation, the obese subjects had an increase in blood vitamin D_3 concentration that was 57% less than the non-obese subjects ($p=0.0042$). This significant difference in the response of the two groups (24 hrs after exposure) is possibly due to an increased sequestration of the synthesized vitamin D_3 in the subcutaneous fat. Thus, the obese subjects had similar precursor (7-DHC) levels and conversion to previtamin D_3 and vitamin D_3 in the skin, but the excess adipose tissue may have reduced the release of vitamin D_3 into the circulation. Adipose tissue, which stores vitamin D_3, is much more available in the obese subjects to sequester this cutaneously synthesized vitamin D_3.

Brock et al. (19) investigated a number of predictors of vitamin D status in a total of 2621 subjects (1357 males, 1264 females aged 55-74) from the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO). They found a significant association ($p<0.001$) between high BMI (≥30) and low vitamin D levels, again explaining this inverse relationship of 25(OH)D and obesity with the "trapping" of vitamin D3 in the extra adipose tissue. Interestingly, the odds ratio was much higher for

females vs. males (3.6 vs. 2.0) when looking at how a BMI >30 predicted vitamin D status.

Konradsen et al. (74) conducted a study of 2187 subjects (410 males, 1777 females, aged 46.8±14.9) recruited from a metabolic and medical lifestyle management clinic in Norway. They observed that as BMI increased there was a significant reduction ($p<0.001$) in serum 25(OH)D concentration. Those that had a BMI >39.9 had serum 25(OH)D levels that were 24% lower than those with a healthy (<25) BMI.

Blum et al.(15) measured adipose tissue and serum vitamin D_3 concentrations in 17 obese subjects and found a moderately strong positive correlation between these measurements (0.68, $p=0.003$). This is consistent with adipose tissue being a storage reservoir for vitamin D_3 (not allowing it to arrive at the liver), thus also seeing low serum 25(OH)D status which is commonly seen in obese subjects.

Fat sequestration of vitamin D_3 is not the only hypothesis for lower vitamin D status among overweight/obese individuals, and Drincic et al. (32) seem to have a much simpler explanation. Utilizing a volumetric dilution model, they concluded that when serum 25(OH)D levels in the overweight/obese individuals are adjusted for body weight/size, there is no longer any difference between overweight/obese and healthy weight individuals in vitamin D status. These authors actually concluded that for vitamin D deficient obese individuals, treatment should be based on body weight and a range of 70-80 IU/kg/day would be needed to produce individuals with sufficient vitamin D status. The likely higher vitamin D requirement in the obese population and the idea that vitamin D intakes should be based on body weight/size has been illustrated

in numerous studies (4, 40, 41, 75, 77). Thus, just knowing someone's baseline vitamin D deficiency status alone is not enough, and BMI should be considered when recommending the dose and/or duration of vitamin D supplementation.

There is also evidence that for overweight/obese individuals, losing weight leads to improved vitamin D status, which could benefit overall health. In a 2 year clinical weight loss trial of overweight/obese women, Rock et al. (99) found that those women who lost ≥ 10% of their baseline body weight increased their 25(OH)D levels by 5 ng/ml vs 1.9 ng/ml for those women who did not lose any weight (P=0.014). Vitamin D deficiency of all participants decreased from 49% to 36%, and only 17% of those that achieved a healthy BMI by the end of the study were considered vitamin D deficient.

It is not fully understood how vitamin D operates in human adipose tissue. Nimitphong et al. showed that VDR and CYP27B1 were expressed in human adipose tissue, preadipocytes, and newly differentiated adipocytes. They hypothesize that active vitamin D hormone is involved in the healthy turnover and remodeling of adipose tissue, aiding in the creation of newly differentiated and more insulin-sensitive adipocytes, thus possibly helping with obesity and Type 2 Diabetes. Further studies are needed to uncover the molecular mechanisms of active vitamin D hormone in adipose tissue (91, 109).

Adipose tissue expressing vitamin D metabolizing enzymes (25-hydroxylase, 1α-hydroxylase (CYP27B1), 24-hydroxylase (catabolic CYP24A1)) and VDR has been demonstrated by Wamberg et al. as they compared this expression between lean and obese individuals. They found decreased expression levels of 25-hydroxylase and

CYP27B1 by 71% ($P<0.0001$) and 49% ($P<0.05$), respectively, in the subcutaneous adipose tissue of the obese subjects. The prevalence of low 25(OH)D levels (<30ng/ml) was very high in the obese subjects compared to the lean subjects (90% vs 50%). Whether 25(OH)D created in adipose tissue contributes to circulating 25(OH)D levels is not currently known, and it is plausible that there could be increased catabolism (via CYP24A1) of vitamin D in obesity. Wamberg et al. also analyzed CYP24A1 in obese subjects before and after a 10% weight loss and found that CYP24A1 expression increased by 79% ($P<0.05$) after weight loss, signifying a possible increased creation and use of $1,25(OH)_2D$ within adipose tissue, followed by its degradation after positive effects (121).

These positive effects of $1,25(OH)_2D$ within adipose tissue are important, as adipose tissue is not just a fatty acid storage reservoir but rather a highly metabolic tissue intimately involved in lipid and glucose metabolism. A variety of hormones and cytokines are produced in adipose tissue, and a chronic inflammatory state along with adipose tissue dysfunction is associated with obesity. An increased secretion of pro-inflammatory cytokines (tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6)) by adipose tissue in obesity can lead to increased inflammation, macrophage infiltration, and increased endothelial adhesion leading to the promotion of atherosclerosis. Vitamin D has anti-inflammatory effects, as an in vitro study by Zhang et al revealed a 25(OH)D dose-dependent, downregulation of IL-6 and TNF-α production in human monocytes. Another in vitro study by Zehnder et al. demonstrated that human endothelial cell synthesis of $1,25(OH)_2D$ is stimulated in response to inflammatory cytokines, illustrating an autocrine response mechanism, possibly

modulating endothelial cell adhesion and decreasing atherosclerosis progression (133, 134).

At the present time it is not fully understood how vitamin D is sequestered and/or mobilized from lipid storage and at what level of adiposity this may occur (18). It seems clear that our current obesity epidemic is contributing to our suboptimal vitamin D levels, as vitamin D deficiency/insufficiency is very common in overweight/obese individuals. With this elevated prevalence of deficient vitamin D status in overweight/obese individuals and its associated comorbidities, regular vitamin D screening in this population seems warranted along with treatment options that are inexpensive and easy to implement (sensible sun exposure and vitamin D supplementation).

Vitamin D and Type II Diabetes

Type II diabetes mellitus (T2DM) has become a serious health care problem, as this disease is exploding in prevalence and contributing to an ever increasing financial crisis in our health care system. T2DM is associated with multiple co-morbidities and increased mortality. As someone ages, how well they control their blood glucose appears to influence their health greatly and helps determine their risk for numerous chronic diseases. New innovative techniques to help manage and prevent T2DM are needed, and vitamin D's role in this is being explored more recently to possibly reduce the burden that T2DM has on its sufferers and on health care costs. T2DM features a number of characteristics, including insulin resistance, altered insulin secretion, and thus hyperglycemia. There are vitamin D receptors (VDR) in the pancreatic β-islet

cells, and thus $1,25(OH)_2D$ helps enhance insulin production and secretion (62). Insulin secretion also requires calcium, so vitamin D may contribute to maintaining proper insulin secretion by helping regulate "extracellular calcium concentrations and flux through cell membranes in the beta cell" (95).

Evidence is accumulating that vitamin D may influence numerous factors that contribute to T2DM. The majority of these studies are epidemiological or cross-sectional, with intervention studies rather scarce. With prevention of T2DM being the ultimate goal, being able to identify easily modifiable risk factors is crucial, as current recommendations (weight loss, improved nutrition) are not being followed adequately and are difficult to maintain for most people, evidenced by the incidence of T2DM increasing rapidly.

Numerous epidemiological studies show an inverse relationship between 25(OH)D levels and a number of glycemic status measures, including fasting plasma glucose, OGTT, and insulin resistance (HOMA-R) (25, 42, 90, 96). Fasting blood glucose is among the number of tests available to measure glycemic status, and is often among the first tests done to check for prediabetes and diabetes. Need et al. (90) studied a large (n=753) group of postmenopausal white women, and on simple correlation found that fasting serum glucose was a negative function of serum 25(OH)D ($p<0.001$). When the subjects were grouped according to their 25(OH)D levels, specifically comparing those with 25(OH)D levels up to 16 ng/ml (deficient vitamin D status) with those above 20 ng/ml, a significantly higher glucose level was found in the vitamin D deficient group ($p<0.001$). The subjects were also divided into groups that

are up to 32 ng/ml (insufficient/deficient) and above 32 ng/ml (sufficient), and a significantly higher fasting serum glucose level was seen in the deficient/insufficient group ($p=0.026$).

Beydoun et al (14) examined a nationally representative sample of US adults (NHANES 2001-04), stratified by central obesity, and examined associations of 25(OH)D with a number of metabolic disturbances (including fasting blood glucose). They found a stronger inverse association of 25(OH)D with fasting blood glucose among the subjects with central obesity (CO+) vs. subjects without central obesity (CO-) ($p<0.001$). With ~2/3 of the US population considered overweight or obese, this is a major concern for vitamin D status in the US. In this study, hypovitaminosis D (25(OH)D < 20 ng/ml) was higher among subjects with CO+ vs. CO- group (37.7% vs. 25.8%, $p<0.0001$). With the associations seen in this study, in order to alleviate certain metabolic disturbances and reduce central obesity, behavioral changes that include improving their vitamin D status would be advisable.

Reiterating the importance of accurate identification of the VDR to try and understand the biological functions of vitamin D, it has been confirmed via the most recent, specific, and sensitive technique that VDR is indeed strongly expressed in pancreatic beta cells (124, 126). The exact function is yet to be determined, but improved insulin secretion from beta cells as well decreased insulin resistance at target tissues are hypothesized. The identification of a VDRE in the human insulin receptor promoter contributed to its proposed role in insulin sensitivity (81). Human vitamin D supplementation studies for Type II Diabetes to date have had some sort of

shortcoming, whether it be too few subjects or too short an intervention. Fortunately there are numerous large scale, randomized controlled trials looking at the role of vitamin D in Type II Diabetes ongoing, so some clarification will hopefully soon arrive when these studies are completed to what exactly is the role of vitamin D in Type II Diabetes prevention and treatment (131).

Vitamin D and Cardiovascular Disease

Numerous cross-sectional studies have shown an association of hypovitaminosis D with a higher prevalence of cardiovascular disease (CVD) and its other risk factors (12, 23, 31, 34, 45, 73, 93, 106, 122, 136). With CVD still the number one cause of death in the US and the prevalence of low vitamin D status, especially among middle-aged and older adults, estimated to be approaching the percentages seen with those who are overweight/obese, there is a definite need to further our understanding of how vitamin D affects cardiovascular health.

There is substantial evidence to suggest that low levels of vitamin D may adversely affect cardiovascular health. Not only is vitamin D associated with many risk factors for CVD, but it may be more directly related, with VDR located on vascular and cardiac cells (61, 122), although these results are now in question due to a more sensitive and specific VDR antibody that did not detect VDR in smooth muscle, heart muscle, or skeletal muscle (125, 126) In addition to the obesity and fasting blood glucose already covered, vitamin D status also affects CV health via its association with hypertension.

Evidence exists of $1,25(OH)_2D$ regulating the major blood-pressure regulating hormone renin in the kidneys. The active vitamin D hormone functions as an inhibitor of the renin-angiotensin system (RAS), which is beneficial as over-activation of RAS can lead to hypertension (78, 108). Burgaz et al (21) determined from a meta-analysis of mostly cross-sectional studies that blood 25(OH)D concentration is inversely associated with hypertension. They found that the OR for hypertension decreased by 16% for every 16 ng/ml increase in blood 25(OH)D concentration. Burgaz et al. looked at a community of elderly men, investigating the prevalence of hypertension in relation to 25(OH)D concentration. They found that men with 25(OH)D concentration of <15 ng/ml had a 3-fold higher prevalence of hypertension compared to men with a concentration ≥15 ng/ml. They also hypothesized that the mechanism is the negative regulation of renin gene transcription via a vitamin D receptor-mediated mechanism (21, 79), and/or VDR and 1α-hydroxylase activity on blood vessel walls (112). To this point there has not been consistency in reducing blood pressure with vitamin D supplementation in randomized controlled trials, so it is premature to recommend vitamin D supplementation for the prevention and treatment of hypertension.

Endothelial stress has been shown to induce the release of a novel growth factor, namely vitamin D binding protein (DBP). If this stress on endothelial cells is consistently excessive, a dangerous situation can arise where normal responses to stress or injury can now become flawed repair processes that lead to high blood pressure, atherosclerosis, and increased risk of cardiovascular events. With a low vitamin D status, DBP is released leading to the migration of vascular smooth muscle cells (VSMCs) to sites of endothelial dysfunction as part of the paracrine response for

vascular remodeling. Phosphate and TNF-α (both increased in low vitamin D status) increase osteogenic processes in VSMCs which may increase the risk for vascular calcification, leading to increased blood pressure and increased risk of cardiovascular events. Vitamin D plays a regulatory role during the endothelial response to injury, as 25(OH)D and $1,25(OH)_2D$ inhibit the growth and migration of VSMCs. Thus when the vitamin D sterols are occupying the binding site (during sufficient vitamin D status) the signals to the VSMCs to proliferate and migrate are not activated, and vitamin D can potentially play a protective role to maintain vascular health (22, 98, 114).

Triglyceride levels are another CV risk factor that has been shown to be associated with low vitamin D status. Martins et al. (82) looked at the prevalence of CV risk factors and 25(OH)D levels in the third NHANES. After adjusting for age, gender, and race, 32.9% of people in the lowest quartile of vitamin D levels (<21 ng/ml) has TG levels of ≥150 mg/dl vs. 23.8% of those in the highest quartile (≥37 ng/ml). This equated to an OR of 1.47 for high TG levels in individuals with low 25(OH)D levels.

High-density lipoprotein levels are now recognized as a risk factor for CV disease, as a level of ≥40 mg/dl or a total/HDL cholesterol ratio <5.0 is recommended to reduce CV disease risk. In the NHANES 2001-2004 data, those that had a total/HDL cholesterol ratio <3.5 (38.7%) had a mean 25(OH)D level of 25.5 ng/ml vs. ≥5.0 (23.9%) had a mean 25(OH)D level of 23.0 ng/ml ($p<0.001$) (44).

There is also an HDL hypothesis that postulates an elevated HDL level increases vitamin D levels, especially when vitamin D levels are typically suppressed (winter). Cholesterol is an important component of the barrier function of the skin, and during

times of stress (winter), more cholesterol is needed. Cholesterol is available either by endogenous synthesis (via 7-DHC) or from the plasma (HDL scavenging for cholesterol). If there is a reduction in cholesterol concentration for barrier function of the skin, then there is an increase in 7-DHC reductase activity (the enzyme that converts 7-DHC to cholesterol). But if you have an influx of cholesterol from your optimal HDL levels and its scavenging activity, then you will have an increased 7-DHC concentration for increased vitamin D synthesis (94).

Overall, components of metabolic syndrome, with its associated risk factors, increase the risk of CVD and diabetes. Vitamin D appears to have an association with all the factors that make up metabolic syndrome, thus it could be advisable to identify vitamin D status as part of the screening process for these risk factors of chronic disease.

Vitamin D and Cognitive Function

Vitamin D's role in cognitive function is very intriguing to many researchers. With the high prevalence of vitamin D deficiency/insufficiency and Alzheimer's and dementia becoming an ever increasing burden in an aging society, this potential link warrants further investigation. A major hypothesis for the cause of Alzheimer's and dementia is the vascular hypothesis, with cognitive dysfunction occurring secondarily to cerebrovascular or cardiovascular disease. These blood vessels are at increased risk of damage with conditions such as endothelial dysfunction, HTN or T2DM present, and vitamin D's role in ameliorating these diseases has been previously discussed. Roman et al clarified a difference between vascular dementia and AD, as executive functions

(planning, problem solving, sequencing) are more profoundly affected than memory impairment (101).

The presence of VDR protein and 1α-hydroxylase and their distribution in the brain has been detected (33), but the antibodies used in previous studies were not VDR-specific, so future studies will be required to confirm results (126). They were previously co-localized in neurons and glial cells throughout parts of the brain most affected by cognitive disorders. This leads to a compelling argument for a functional role for vitamin D in the human brain (20).

With aging, due to decreasing thickness of the skin and the reduction in the 7-DHC content, the ability of the skin to synthesize vitamin D significantly decreases. MacLaughlin and Holick saw a >50% decrease in previtamin D_3 production when comparing individuals in their 70's vs teens. While aging does not seem to negatively affect intestinal absorption of vitamin D, an increased incidence of non-alcoholic fatty liver disease (NAFLD) and age related functional decline of the kidneys can potentially has an adverse effect on the hydroxylation reactions that must occur to create the circulating and active forms of vitamin D (39, 80). Combine this with low vitamin D dietary intake and limited sun exposure and it becomes clear why vitamin D deficiency is common in the elderly. Older adults with Alzheimer's disease (AD) have a very high prevalence, ~70-90%, of vitamin D deficiency (11, 61).

With evidence of the importance of sufficient vitamin D status for proper blood pressure control and endothelial function, Gorelick et al. (48) highlighted the importance of blood pressure control in middle-aged individuals for the prevention of

dementia later on in life. The evidence is much more established for lowering blood pressure in mid-life rather than waiting until you are over the age of 80 for the prevention of dementia. Being proactive about one's vascular health and lowering blood pressure in patients with no cognitive impairment will reduce the risk of future cognitive problems.

There are a number of potential neuroprotective roles of vitamin D in regards to AD. Active vitamin D ($1,25(OH)_2D$) exerts its neurosteroid type actions through VDRs, which are present in neuronal and glial cells all throughout essential cognitive regions of the brain, including the hippocampus, hypothalamus, and cortex. When $1,25(OH)_2D$ binds to VDR, this triggers neuronal protection via anti-inflammatory action, as AD is also an inflammatory brain disease with microglia located near the β-amyloid (Aβ) plaques and increased release of TNF-α seen in several studies (16, 49). As previously discussed, vitamin D downregulates TNF-α production and promotes cytokines and macrophages to increase Aβ clearance. In addition, $1,25(OH)_2D$-VDR complex stimulates neurotrophic agents, reduces Aβ42 peptide accumulation via Aβ peptide phagocytosis, and increases brain-to-blood Aβ efflux at the blood brain barrier. Finally, vitamin D regulates expression of various neurotransmitters in the brain, including acetylcholine, dopamine, and serotonin. All of these neurosteroid characteristics of vitamin D may help to address cognitive decline in older adults (9, 43, 103).

Scott et al. (105) analyzed data from the Nutrition and Memory in Elderly study (NAME). After subjects completed a full neurological and psychiatric examination

along with an MRI, they found lower vitamin D concentrations (29.5 ng/ml vs. 16.5 ng/ml; p=0.03) in patients with dementia than those without. Also, using the cutoff of 20 ng/ml, those below were associated with a higher prevalence of a possible or probable AD diagnosis (17.1% vs. 6.9%; $p<0.01$).

A main component of Alzheimer's disease is defective clearance of Aβ protein. Masoumi et al.(83) combined $1,25(OH)_2D$ with curcuminoids and stimulated macrophages to observe its actions on Aβ. The result was that $1,25(OH)_2D$ stimulated Aβ phagocytosis and clearance. Maintaining sufficient vitamin D status may decrease neuronal cell death and decrease the risk for Alzheimer's disease.

With an aging population and the growing health care costs as well as the financial and emotional burden of caring for the elderly, investigating easily implementable strategies to modulate risk factors and the pathology of cognitive dysfunction make sense. Vitamin D fits this criteria, and maintaining an adequate vitamin D status may prove to be a cost-effective intervention in helping to delay cognitive dysfunction.

CHAPTER III

METHODS

Purpose

The purpose of the present study was to investigate the relationship between current vitamin D status and risk factors (components of metabolic syndrome and cognitive dysfunction) for numerous chronic diseases (cardiovascular disease, diabetes, dementia) in men and women aged 50-70 years. This study aimed to determine if deficient and/or insufficient vitamin D status was associated with risk factors (components of metabolic syndrome and cognitive dysfunction) for chronic disease (cardiovascular disease, diabetes, cognitive dysfunction).

Subjects

Eighty-eight (88) recreationally active older individuals aged 50-70 years old were enrolled in this study. One subject was realized to be age 72 after consenting, after which she was withdrawn from the study without any data collected. Fifteen subjects completed the initial visit but did not return for the testing visit, as they did not respond to follow-up contact from the investigator. Seventy-two (72) Caucasian recreationally active older individuals (54 females, 18 males) aged 50-70 years old completed this study. Each subject completed the written informed consent along with a food frequency questionnaire and sun exposure questionnaire prior to participation in the study. Participants volunteered from the Norman, OK (USA) area and surrounding communities.

Recruitment

Subjects were recruited through word of mouth, emails, and flyers. Flyers were posted on the campus, as well as the prominent public places in and around Norman-Oklahoma City metropolitan area.

Each volunteer read and signed a written informed consent form approved by the Institutional Review Board (IRB) at the University of Oklahoma, Norman.

Inclusion Criteria

1. Men and women aged 50-70 years old.
2. Subjects weighed no more than 300 lbs. which was the weight limit of the DXA machine.
3. Subjects had no cognitive problems that can interfere with their participation. Subjects must score greater than or equal to 27 points (out of 30) on the Mini Mental State Examination (MMSE) to indicate a normal cognition.
4. Subjects were able to speak and understand English.

Exclusion Criteria

1. Individuals < 50 years old or > 70 years old.
2. Subjects weighing more than 300 lbs.
3. Subjects with cognitive impairment that did not allow them to complete the current process or testing. Subjects scoring less than 27 points (out of 30) on the MMSE.

Research Design

This study used a cross-sectional research design. All subjects completed an IPAQ questionnaire to estimate their sedentary/ active status. They also filled out a medical history, food frequency, and sun exposure questionnaire. No intervention took place and data was collected during the spring/fall semester of 2013. Eighty-eight (88) males and females aged 50-70 were recruited from the city of Norman, OK and surrounding communities and were consented and screened (questionnaires) for participation. One subject was realized to be age 72 after consenting, after which she was withdrawn from the study without any data collected. Fifteen subjects completed the initial visit but did not return for the testing visit, as they did not respond to follow-up contact from the investigator. Seventy-two (72) recreationally active older individuals (54 females, 18 males) aged 50-70 years old completed this study.

Bone Mineral Density Measurements

Dual Energy X-ray Absorptiometry (DXA, GE Lunar Prodigy, Prodigy encore software version 10.50.086, Madison, WI) assessed Total Body BMD (g/cm^2) and a total body scan was performed in order to obtain % body fat and android/gynoid ratio (A/G Ratio). The DXA machine was calibrated on each testing day prior to scanning subjects, and the scanning procedures were standardized for all participants. Subjects dressed in light clothing with no attenuating materials (e.g., metal). Subjects were placed in a supine position on the DXA table, arms were placed close to the sides of the body, and Velcro straps were placed around the knees and ankles to hold the legs together during the scans. If the head of the subject did not appear within 2-3 sweeps of

the scan images, the scan was aborted for a new measurement after the subject had been repositioned. A single technician performed all scans and analysis. The lab %CV for the total body BMD was 0.6% and for % body fat was 1.87%.

Body Composition

Body composition was calculated from the total body scan to obtain % body fat and A/G Ratio. Compared % body fat to norms of 15% for males and 25% for females. A/G Ratio > 1 for males and > 0.8 for females = increased chronic disease risk.

Body Measurements

Height was measured to the nearest 0.5 cm using a wall mounted stadiometer (Stadi-O-Meter, Novel Products Inc., Rockton, IL) without shoes. The participant placed their heels together against the wall and was asked to stand up tall with back flat against the wall and their head aligned in the sagittal plane. Weight was measured to the nearest 0.1 kg using a digital bodyweight scale (TANITA BWB-800, TANITA, Japan) with the participant shoeless and wearing light weight clothing. Body mass index (BMI) was calculated as body mass in kilograms divided by height in meters squared (kg/m2). Waist circumference was measured to the nearest cm using a tape measure, at the top of the hip bone, level with the navel.

Physical Activity

To predict the status of an individual, the International Physical Activity Questionnaire (IPAQ) was used. The IPAQ has three categorical scores (low, moderate, high) for differentiating individuals on the basis of their physical activity (PA). It asks about the time you have spent being physically active in the last 7 days. There are 5 parts: 1) Job related PA; 2) Transportation PA; 3) Housework, maintenance, caring for

family; 4) Recreation, sport, leisure-time PA; 5) Time spent sitting. High PA: 7 days/wk walking or moderate intensity activity accumulating at least 3000 MET-minutes/wk or 3 days of vigorous activity and accumulating at least 1500 MET-minutes/wk. Moderate PA: 3 days of vigorous activity of at least 20 min per day or 5 days of walking or moderate intensity activity for at least 30 min per day or 5 or more days of any combination of walking, moderate-intensity, or vigorous-intensity activities achieving at least 600-MET-min/wk. Low PA: Those individual that do not meet the criteria for moderate or high PA. This is the lowest level of PA.

Sun Exposure

A sun exposure questionnaire was given to help assess sunlight exposure and illuminate the role of cutaneous vitamin D synthesis in vitamin D status. A sun exposure recall questionnaire previously used by Hanwell et al (52) utilized 3 components: 1) Sun Exposure Score (sum of the daily products of Time Outdoors and Skin Exposure); 2) Time Outdoors: 0 = <5 min; 1 = 5-30 min; 3 = > 30 min; 3) Amount of Skin Exposed: 1 = hands and face; 2 = hands, face, arms; 3 = hand, face, legs; 4 = bathing suit. Range of the Sun Exposure Score is 0-56.

Blood Pressure (BP)

Brachial systolic (SBP) and diastolic BP (DBP) were measured using an automatic blood pressure measuring device (Omron IntelliSense Automatic Blood Pressure Monitor with Easy Wrap Cuff, model HEM-773AC, Vernon Hills, IL). Two measurements were taken one minute apart on the right arm and averaged. If these measurements were not within 5 mmHg, a third measurement was taken and used for analysis.

Pulse Wave Analysis (PWA)

Applanation tonometry (SphygmoCor, AtCor Medical, Sydney, Australia) and a high-fidelity strain-gauge transducer (Miller Instruments, Houston, TX, USA) was used to obtain pressure waveforms at the radial artery on the right arm. Aortic blood pressure waveforms were derived from radial waveforms using a generalized validated transfer function (SphygmoCor, AtCor Medical, Sydney, Australia) to obtain measures of aortic blood pressure, arterial stiffness, and wave reflection. Two measurements with an operator index > 80 were obtained and the measurement with a higher operator index was used for analysis.

Nutrient Analysis

FoodWorks (The Nutrition Company, Long Valley, NJ) nutrient analysis software was utilized to analyze a 3-day food record (2 weekdays, 1 weekend day) for CHO, PRO, FAT, Calcium, and Vitamin D intake. In addition, a food frequency questionnaire (Ca/Vitamin D) to assess vitamin D supplementation intake was administered.

Blood Sampling

Fasting blood samples were taken by trained personnel by venipuncture. A lipid panel (Cholesterol, Triglycerides, HDL, LDL (calculated) Cholesterol/HDL Ratio (calculated)), Blood Glucose, and Vitamin D were measured.

Vitamin D Assay

Vitamin D status was determined by Immunochemiluminometric assay (ICMA). This assay is performed on the DiaSorin LIAISON instrument by LabCorp. Vitamin D sufficiency was defined as greater than or equal to 30 ng/ml.

Cognitive Function

In cooperation with the Cognitive Science Research Center and the Center for the Study of Human Operator Performance (C-SHOP), the Automated Neuropsychological Assessment Metrics ($ANAM_4$) test system was utilized to measure cognitive function. A specific battery of tests was given in a specific order and the results were correlated with vitamin D status. The following $ANAM_4$ test order (with rationale and description) (5) was implemented:

1) Simple Reaction Time
    - Cognitive Domain – "Results of this test are used as an index of attention (i.e., reaction time & vigilance) and visuo-motor response timing."
    - Test Description – "This test measures simple reaction time by presenting the user with a series of "*" symbols on the display. The user is instructed to respond as quickly as possible by pressing a button each time the stimulus appears."
2) Code Substitution – Learning
    - Cognitive Domain – "Results of this test are used as an index of complex scanning, visual tracking, and attention."

- Test Description – “In this test the user must compare a displayed digit-symbol pair with a set of defined digit-symbol pairs, or the key. The user presses designated buttons to indicate whether the pair in question represents a correct or incorrect mapping relative to the key. In the Learning phase (simultaneous presentation mode), the defined pairs are presented on the screen along with the digit-symbol pair in question.”

3) Procedural Reaction Time

- Cognitive Domain – “This test measures the reaction time and processing efficiency associated with following a simple set of mapping rules.”
- Test Description – “There are three possible blocks of trials for this test. In the Basic Block, the user is presented with a number constructed on the display using a large dot matrix (either a 2, 3, 4, or 5). The user is instructed to press one designated button for a "low" number (2 or 3) and another designated button for a "high" number (4 or 5). In the Coded Block, the user is presented with the same numbers and mapping rules, but the numbers are visually distorted by the presence of noise in the matrix and are more difficult to read. In the Time-Uncertainty Block, the user is presented with the same undistorted stimuli and mapping rules as in the Basic Block, but at longer, irregular interstimulus intervals.”

4) Mathematical Processing

- Cognitive Domain – “Results of this test are used as an index of basic computational skills, concentration, and working memory.”

- Test Description – “During this task, an arithmetic problem involving three single-digit numbers and two operators is displayed (e.g., "5 - 2 + 3 ="). The user presses buttons to indicate whether the answer to the problem is less than five or greater than five.”

5) Matching to Sample
    - Cognitive Domain – “Results of this test are used as an index of spatial processing and visuo-spatial working memory.”
    - Test Description – “During this test the user views a pattern produced by eight shaded cells in a 4x4 sample grid. The sample is then removed and two comparison patterns are displayed side by side. One grid is identical to the sample grid and the other grid differs by one shaded cell. The user is instructed to press a designated button to select the grid that matches the sample.”
6) 2-Choice Reaction Time
    - Cognitive Domain – “Results of this test are used as a measure of processing speed and alternating attention with a motor speed component.”
    - Test Description – “This test measures choice reaction time by presenting the user with a "*" or "o" on the display. The user is instructed to respond as quickly as possible by pressing the designated button for each stimulus as soon as the stimulus appears. This test can be modified to present alternative symbols as stimuli.”
7) Code Substitution – Delayed

- Cognitive Domain – "This test provides a measure of learning and delayed visual recognition memory."
- Test Description – "In this test the user is presented with a digit-symbol pair and must decide from memory if this pairing is correct based on the key presented during the Code Substitution — Learning test taken earlier in the test battery. The user presses designated buttons to indicate whether the pair in question represents a correct or incorrect match based on the earlier presented key."

This ANAM test gave a broad spectrum of analysis of cognitive processing in a very user friendly, efficient (~25 min) manner. These tests utilized common pointing devices (mouse) and required minimal learning to master.

Data Analysis

All data were reported as mean ± standard error (SE). Data analysis was conducted using SPSS 19.0 (SPSS Inc., Chicago, IL) software, including descriptive statistics and 2 way ANOVA (PA, Gender) to determine between group differences for all outcome measures based on physical activity and gender. Pearson correlation coefficients were used to explore potential relationships between serum 25(OH)D levels and disease risk factors. A Chi-Square analysis involving VITD Status and Gender was conducted to examine the association and the sampling distribution between these two variables. A Chi-Square analysis involving VITD Status and Physical Activity Level was conducted to examine the association and the sampling distribution between these two variables. A one-way ANOVA was conducted to evaluate potential differences

across the three levels of vitamin D status (Deficient, Insufficient, Sufficient) for each risk factor for chronic disease. Next, data was split based on Gender and Physical Activity Level. A one-way ANOVA was conducted to evaluate potential differences across the three levels of vitamin D status (Deficient, Insufficient, Sufficient) for each Throughput score on the cognitive tests. A Chi-Square analysis involving VITD Status and ANAM score (Average=all 7 tests scored average; Below Average=at least 1 test scored below average) was conducted to examine the association and the sampling distribution between these two variables. A Chi-Square analysis involving VITD Status and VITD Synthesis was conducted to examine the association and the sampling distribution between these two variables. The level of significance for all analyses was set at α=0.05.

CHAPTER IV

RESULTS & DISCUSSION

Purpose

The purpose of the present study was to investigate the relationship between current vitamin D status and risk factors (components of metabolic syndrome and cognitive dysfunction) for numerous chronic diseases (cardiovascular disease, diabetes, dementia) in men and women aged 50-70 years. This study aimed to determine if deficient and/or insufficient vitamin D status was associated with risk factors (components of metabolic syndrome and cognitive dysfunction) for chronic disease (cardiovascular disease, diabetes, cognitive dysfunction).

Results

For each set of outcome variables, the data was first analyzed for the entire group (n=72) and then a two-way ANOVA examined the main effects of gender and physical activity levels on the outcome variables. After a brief discussion of the group findings and the influence of gender on the variables, a separate table and discussion follows that examines the influence of physical activity on the variables.

Participant Characteristics

On average, participants as a group, were overweight/obese based on their BMI (27.2) and % BF (38.2%) measures. These numbers put individuals at increased risk for chronic disease. Healthy BMI is 18.5-24.9 kg/m^2, and while it is a tool to diagnose obesity, keep in mind that BMI does not distinguish between lean and fat mass. The men averaged 29.1% BF and women 41.1% BF in this study, which categorizes both as obese, and well above the normal % BF for this population, which has an upper limit of

25% BF for females and 15% BF for males (1). It appeared as though % BF tended to decline with increased PA, and 86% self-reported that they engaged in at least moderate physical activity on a regular basis, so that is encouraging for those obese individuals knowing that with hard work they can expect some positive body composition changes. A two-way ANOVA (Gender x Physical Activity Level) detected a significant main effect for gender as men and women differed significantly ($p<0.05$) in the following characteristics: 1) Age ($p=0.017$); 2) standing height ($p=0.000$); 3) weight ($p=0.006$); 4) % body fat ($p=0.000$); 5) android/gynoid (A/G) ratio ($p=0.000$) (Table 1). Men on average were older (+3.5yrs), taller (+15.5cm), heavier (+12kg), and had a larger A/G Ratio (1.2 vs .95) compared to women, who themselves had a higher %BF (+12.1%) compared to men. With increasing PA there was an improvement in most body composition measures, especially for the women. No interaction ($p<0.05$) (Gender x PA) was detected for any of these body composition variables.

Table 1. Participant Characteristics

Group	N	Age	Ht. (cm)	Wt. (kg)	BMI	%BF	A/G Ratio	WCcm
Total	72	60.1 (0.6)	166.4 (1.1)	75.4 (1.9)	27.2 (0.6)	38.2 (1.1)	1.02 (.02)	96.0 (1.6)
Men	18	62.7 (1.0)	178.0 (1.7)	84.4 (3.1)	26.6 (0.8)	29.1 (1.4)	1.20 (.03)	98.8 (2.2)
Low PA	0	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Mod PA	10	62.4 (1.5)	175.6 (2.2)	82.7 (5.3)	26.7 (1.4)	29.2 (2.0)	1.19 (.04)	98.4 (3.6)
High PA	8	63.1 (1.5)	181.0 (2.5)	86.6 (2.5)	26.4 (0.6)	29.0 (1.9)	1.21 (.06)	99.2 (2.1)
Women	54	59.2 (0.7)*	162.5 (0.9)*	72.4 (2.2)*	27.4 (0.8)	41.2 (1.1)*	0.95 (.02)*	95.1 (1.9)
Low PA	10	59.3 (1.6)	161.2 (2.2)	80.9 (7.7)	30.9 (2.7)	46.2 (2.2)	0.98 (.04)	103.4 (5.8)
Mod PA	23	59.1 (1.1)	163.8 (1.5)	76.0 (2.9)	28.5 (1.1)	43.4 (1.6)	0.97 (.03)	98.1 (2.6)
High PA	21	59.2 (1.3)	162.3 (1.3)	64.5 (2.0)	24.5 (0.8)	36.5 (1.1)	0.93 (.04)	87.8 (2.4)

Data presented at mean (SE); BMI, body mass index; %BF, percentage body fat; A/G Ratio, android/gynoid ratio; WCcm, waist circumference in centimeters. *$p<0.05$ vs. Men.

A two-way ANOVA detected a significant main effect for physical activity as a significant difference ($p<0.05$) was found between the different physical activity levels for BMI ($p=0.019$), % BF ($p=0.006$), and WCcm ($p=0.028$). Least Significant Difference (LSD) post-hoc analysis revealed a significant decrease for BMI between Low PA and High PA ($p=0.006$); a significant decrease for % BF between Low PA and High PA ($p=0.000$) and between Low PA and Moderate PA ($p=0.000$); a significant decrease for WCcm between low PA and high PA ($p=0.039$). BMI, %BF, and WCcm all decreased significantly with increased physical activity. BMI mean values went from an obese categorization to healthy when going from low PA to high PA, along with %BF and WCcm being significantly lower, 11.8% and 12.4 cm respectively (Table 2).

Table 2. Participant Characteristics at Different Physical Activity Levels

Group	N	Age	Ht. (cm)	Wt. (kg)	BMI	%BF	A/G Ratio	WCcm
Total	72	60.1 (0.6)	166.4 (1.1)	75.4 (1.9)	27.2 (0.6)	38.2 (1.1)	1.02 (.02)	96.0 (1.6)
Low PA	10	59.3 (1.6)	161.2 (2.2)	80.9 (7.7)	30.9 (2.7)	46.2 (2.2)	0.98 (.04)	103.4 (5.8)
Mod PA	33	60.1 (0.9)	167.1 (1.6)	78.0 (2.6)	28.0 (0.9)	39.1** (1.7)	1.03 (.03)	98.2 (2.1)
High PA	29	60.3 (1.0)	167.4 (2.0)	70.6 (2.5)	25.0* (0.6)	34.4* (1.1)	1.01 (.04)	91.0* (2.1)

Data presented at mean (SE); BMI, body mass index; %BF, percentage body fat; A/G Ratio, android/gynoid ratio; WCcm, waist circumference in centimeters. *$p<0.05$ vs. low PA. **$p<0.05$ vs. low PA.

A Chi-square analysis involving VITD_Status and Gender was conducted to examine the association and the sampling distribution between these two variables (Table 3). The association between the two variables was significant ($p=0.008$) and the results revealed that while exactly 50% (36 out of 72) of the study population was vitamin D deficient and/or insufficient, a higher percentage of males (12 out of 18 = 66.6%) were vitamin D deficient and/or insufficient than females (24 out of 54 = 44.4%). In addition, a Chi-square analysis involving VITD_Status and Physical Activity Level was conducted to examine the association and the sampling distribution between these two variables (Table 4). The association between the two variables was significant ($p=0.002$) and the results revealed that 70% (7 out of 10) of the low PA group, 51.5% (17 out of 33) of the moderate PA group, and 41.4% (12 out of 29) of the high PA group were vitamin D deficient and/or insufficient.

Table 3. Chi-Square Analysis

VITD_Status * GENDER Crosstabulation (p=0.008)					
			GENDER		Total
			MALE	FEMALE	
VITD_Status	Deficient	Count	0	9	9
		% within VITD_Status	0.0%	100%	100.0%
		% within GENDER	0.0%	16.7%	12.5%
		% of Total	0.0%	12.5%	12.5%
	Insufficient	Count	12	15	27
		% within VITD_Status	44.4%	55.6%	100.0%
		% within GENDER	66.7%	27.8%	37.5%
		% of Total	16.7%	20.8%	37.5%
	Sufficient	Count	6	30	36
		% within VITD_Status	16.7%	83.3%	100.0%
		% within GENDER	33.3%	55.6%	50.0%
		% of Total	8.3%	41.7%	50.0%
Total		Count	18	54	72
		% within VITD_Status	25.0%	75%	100.0%
		% within GENDER	100.0%	100.0%	100.0%
		% of Total	25%	75%	100%

Table 4. Chi-Square Analysis

VITD_Status * Physical Activity (PA) Crosstabulation (p=0.002)						
			PHYSICAL ACTIVITY			Total
			LOW	MODERATE	HIGH	
VITD_Status	Deficient	Count	5	1	3	9
		% within VITD_Status	55.6%	11.1%	33.3%	100.0 %
		% within PA	50.0%	3.0%	10.3%	12.5%
		% of Total	6.9%	1.4%	4.2%	12.5%
	Insufficient	Count	2	16	9	27
		% within VITD_Status	7.4%	59.3%	33.3%	100.0 %
		% within PA	20.0%	48.5%	31.0%	37.5%
		% of Total	2.8%	22.2%	12.5%	37.5%
	Sufficient	Count	3	16	17	36
		% within VITD_Status	8.3%	44.4%	47.2	100.0 %
		% within GENDER	30.0%	48.5%	58.6%	50.0%
		% of Total	4.2%	22.2%	23.6	50.0%
Total		Count	10	33	29	72
		% within VITD_Status	13.9%	45.8%	40.3	100.0 %
		% within PA	100.0%	100.0%	100.0%	100.0 %
		% of Total	13.9%	45.8%	40.3%	100%

Lipid Panel, Glucose, & Vitamin D

On average, as a group, the participants had normal lipid values with the exception of high LDL cholesterol (109.6 ±3.7), which remained high through all PA levels. They also had high HDL cholesterol (64.8 ± 2.0), which is considered a negative risk factor for CHD when HDL-C > 59 mg/dl. Exercise has been shown to raise HDL levels, and both males and females increased HDL levels with increased PA. TGs also decreased with increased PA, and high TG levels, which have been shown in the literature to be associated with low vitamin D status, are associated with the lowest

vitamin D levels in this study. Both TGs and circulating vitamin D levels (25(OH)D) were lowest in the low PA group. Vitamin D levels as a group were sufficient (>30ng/ml) (31.1ng/ml) and increased with PA, especially for women going from insufficient to sufficient status. Glucose levels as a group were normal and decreased with increasing PA. Two-way ANOVA detected no significant main effect ($p<0.05$) for gender and no significant interaction ($p<0.05$) (Gender x PA) for the lipid panel, GLU, or VIT D (Table 5).

Table 5. Participant Blood Values

Group	N	T Chol	TG	HDL	LDL	GLU	VIT D
Total	72	196.9 (4.2)	116.4 (11.5)	64.8 (2.0)	109.6 (3.7)	95.5 (3.4)	31.1 (1.3)
Men	18	196.7 (9.0)	105.8 (8.9)	62.1 (3.4)	113.4 (7.4)	97.6 (3.1)	28.3 (1.3)
Low PA	0	n/a	n/a	n/a	n/a	n/a	n/a
Mod PA	10	187.1 (12.4)	58.3 (4.6)	58.3 (4.6)	106.7 (10.2)	99.2 (5.3)	27.5 (3.3)
High PA	8	208.6 (12.5)	66.8 (5.0)	66.8 (5.0)	121.9 (10.9)	95.5 (2.7)	29.3 (3.7)
Women	54	196.9 (4.8)	120.0 (15.0)	65.7 (2.4)	108.3 (4.3)	94.8 (4.4)	32.0 (1.7)
Low PA	10	193.1 (9.3)	176.7 (75.4)	61.6 (7.1)	101.1 (7.9)	117.8 (21.8)	23.0 (3.3)
Mod PA	23	198.1 (7.8)	119.0 (10.1)	62.1 (3.3)	112.2 (5.8)	88.2 (1.3)	34.8 (2.2)
High PA	21	197.5 (7.9)	94.1 (9.2)	71.6 (3.6)	107.1 (8.3)	91.2 (3.2)	33.3 (2.3)

Data presented as mean (SE); T Chol (mg/dl), total cholesterol; TG (mg/dl), triglycerides; HDL (mg/dl), high density lipoprotein; LDL (mg/dl), low density lipoprotein; GLU (mg/dl), glucose; Vit D (ng/ml), vitamin D (25(OH)D).

Two-way ANOVA detected a significant main effect for physical activity level as a significant difference ($p<0.05$) was found between the different physical activity levels for GLU ($p=0.018$) and VIT D ($p=0.014$). LSD post-hoc analysis revealed a

significant decrease for GLU between low PA and moderate PA (p=0.020) and between low PA and high PA (p=0.022), with lower GLU levels with increased PA; a significant increase for VIT D between low PA and moderate PA (p=0.038) and between low PA and high PA (p=0.040), with higher VIT D levels with increased PA. Being at least moderately physically active tended to significantly decrease fasting glucose levels and increase circulating vitamin D levels compared to the low PA group.

Table 6. Participant Blood Values at Different Physical Activity Levels

Group	N	T Chol	TG	HDL	LDL	GLU	VIT D
Total	72	196.9 (4.2)	116.4 (11.5)	64.8 (2.0)	109.6 (3.7)	95.5 (3.4)	31.1 (1.3)
Low PA	10	193.1 (9.3)	176.7 (75.4)	61.6 (7.1)	101.1 (7.9)	117.8 (21.8)	23.0 (3.3)
Mod PA	33	194.8 (6.6)	116.4 (7.7)	61.0 (2.6)	110.6 (5.0)	91.5 (2.0)*	31.2 (2.0)*
High PA	29	200.6 (6.6)	95.7 (7.8)	70.3 (2.9)	111.2 (6.7)	92.4 (2.5)*	31.3 (2.2)*

Data presented as mean (SE); T Chol, total cholesterol; TG, triglycerides; HDL, high density lipoprotein; LDL, low density lipoprotein; GLU, glucose; VIT D, vitamin D. *p<0.05 vs. low PA.

Total Caloric and Macronutrient Intakes

Three-day average total caloric intake and macronutrient percentages of total caloric intake for participants are presented in Table 7. Men and women were similar in all characteristics, with increased kcal intake with increased PA, and % kcal from FAT (34.4%) near the upper edge of the recommended range (35%). Two way ANOVA detected no significant main effect (p<0.05) for gender and no significant interaction (p<0.05) (Gender x PA) for kcal and macronutrient intake. As a total group the subjects met the Acceptable Macronutrient Distribution Range (AMDR) for CHO: 45-65% of

total kcals (47.5%); PRO: 10-35% of total kcals (17.9%); and FAT: 20-35% of total kcals (34.4%). Fiber (AMDR = 30g for males 50-70 yrs; 21g for females 50-70 yrs) intake for men was low compared to the AMDR across all PA levels, while fiber intake for women was low in just the low PA category.

Table 7. Three day average total caloric intake, percentages of total daily caloric intake, and dietary fiber intake

Group	N	Kcals	% Kcal Fat	% Kcal PRO	% Kcal CHO	Fiber (g)
Total	72	1833.3 (76.8)	34.4 (1.0)	17.9 (0.6)	47.5 (1.1)	21.7 (1.2)
Men	18	2021.9 (151.3)	32.6 (1.8)	18.5 (1.3)	49.0 (1.7)	22.9 (2.5)
Low PA	0	n/a	n/a	n/a	n/a	n/a
Mod PA	10	1959.4 (244.5)	31.7 (1.8)	17.2 (1.7)	52.7 (1.8)	25.7 (3.5)
High PA	8	2100.1 (165.4)	33.8 (3.4)	20.2 (1.7)	44.4 (2.4)	19.5 (3.4)
Women	54	1770.4 (88.2)	35.0 (1.2)	17.7 (0.6)	47.0 (1.3)	21.3 (1.4)
Low PA	10	1289.8 (98.4)	35.2 (2.4)	17.6 (1.5)	45.4 (2.6)	15.9 (2.9)
Mod PA	23	1878.0 (134.0)	36.7 (2.2)	18.1 (0.9)	46.7 (2.3)	21.7 (1.7)
High PA	21	1881.5 (150.1)	33.1 (1.6)	17.3 (1.1)	48.1 (1.9)	23.4 (2.7)

Data presented as mean (SE); Kcals, calories; % Kcals, % of daily calories from nutrient.

Two-way ANOVA detected a significant main effect ($p<0.05$) for physical activity levels as a significant difference ($p<0.05$) was found between the different physical activity levels for Kcals ($p=0.015$). LSD post-hoc analysis revealed a significant increase for Kcals between low PA and moderate PA ($p=0.024$) and between low PA and high PA ($p=0.017$), as kcals increased with an increase in physical activity, which is typical as you need more kcals to fuel an active lifestyle so that you are not burning muscle for energy (Table 8).

Table 8. Three day average total caloric intake, percentages of total caloric intake, and dietary fiber intake at different physical activity levels

Group	N	Kcals	% Kcal Fat	% Kcal PRO	% Kcal CHO	Fiber (g)
Total	72	1833.3 (76.8)	34.4 (1.0)	17.9 (0.6)	47.5 (1.1)	21.7 (1.2)
Low PA	10	1289.8 (98.4)*	35.2 (2.4)	17.6 (1.5)	45.4 (2.6)	15.9 (2.9)
Mod PA	33	1902.6 (117.2)	35.2 (1.6)	17.8 (0.8)	48.5 (1.8)	22.9 (1.6)
High PA	29	1941.8 (117.8)	33.3 (1.4)	18.1 (1.0)	47.1 (1.6)	22.3 (2.2)

Data presented as mean (SE); Kcals, calories; % Kcals, % of daily calories from nutrient.*$p<0.05$ vs. moderate PA and high PA.

Systemic Hemodynamics

Two-way ANOVA detected no significant main effect for gender for brachial BP and HR measures (peripheral systolic blood pressure (P_SP): p=0.160; peripheral diastolic blood pressure (P_DP): p=0.497; peripheral mean arterial pressure (P_MEANP): p=0.930; heart rate (HR): p=0.528). No main interaction effect (Gender x PA) was detected for any of the brachial BP and HR measures (P_SP: p=0.639; P_DP: p=0.419; P_MEANP: p=0.628; HR: p=0.339 (Table 9). In addition, there was no significant main effect for physical activity levels for any hemodynamic variables. (P_SP: p=0.977; P_DP: p=0.702; P_MEANP: p=0.940; HR: p=0.244) (Table 10). These brachial BP and HR values are similar to normal values (120/80) you want to see for men and women in this age group.

Table 9. Systemic Hemodynamics

Group	N	P_SP	P_DP	P_MeanP	HR
Total	72	123.0 (1.6)	78 (0.9)	94.5 (1.1)	65.6 (1.1)
Men	18	126.6 (2.4)	78.4 (1.4)	94.1 (1.7)	63.4 (2.0)
Low PA	0	n/a	n/a	n/a	n/a
Mod PA	10	126.1 (3.5)	77.6 (1.7)	93.8 (2.3)	62.9 (3.0)
High PA	8	127.1 (3.5)	79.5 (2.4)	94.5 (2.7)	64.1 (2.6)
Women	54	121.2 (1.9)	77.6 (1.1)	94.6 (1.4)	66.3 (1.4)
Low PA	10	120.9 (3.8)	79.3 (1.8)	95.3 (2.4)	70.7 (2.5)
Mod PA	23	122.5 (3.4)	77.9 (1.8)	95.3 (2.4)	67.2 (2.0)
High PA	21	120.0 (2.7)	76.4 (1.6)	93.4 (2.0)	63.2 (2.4)

Data presented as mean (SE); P_SP (mmHg), peripheral systolic blood pressure; P_DP (mmHg), peripheral diastolic blood pressure; P_MEANP (mmHg), peripheral mean arterial pressure; HR (bpm), heart rate.

Table 10. Systemic Hemodynamics at Different Physical Activity Levels

Group	N	P_SP	P_DP	P_MeanP	HR
Total	72	123.0 (1.6)	78 (0.9)	94.5 (1.1)	65.6 (1.1)
Low PA	10	120.9 (3.8)	79.3 (1.8)	95.3 (2.4)	70.7 (2.5)
Mod PA	33	123.6 (2.6)	77.8 (1.4)	94.9 (1.8)	65.9 (1.7)
High PA	29	122.0 (2.2)	77.2 (1.4)	93.7 (1.6)	63.5 (1.9)

Data presented as mean (SE); P_SP (mmHg), peripheral systolic blood pressure; P_DP (mmHg), peripheral diastolic blood pressure; P_MEANP (mmHg), peripheral mean arterial pressure; HR (bpm), heart rate.

Central Hemodynamics

Two-way ANOVA detected a significant main effect (p=0.05) for gender for heart rate corrected central augmented pressure (C_AP_HR75) (p=0.000) and augmentation index (C_AGPH_HR75) (p=0.000). These measures of arterial stiffness were much higher in the women, which indicates increased arterial stiffness compared to the men. No significant interaction (Gender x PA) was detected (C_AP_HR75: p=0.833; C_AGPH_HR75: p=0.737; central systolic pressure (C_SP): p=0.773; central diastolic pressure (C_DP): p=0.434) (Table 11). No significant main effect was

detected for physical activity on measures of central hemodynamics (C_AP_HR75: p=0.713; C_AGPH_HR75: p=0.309; C_SP: p=0.925; C_DP: p=0.725) (Table 12).

Table 11. Central Hemodynamics

Group	N	C_AP_HR75	C_AGPH_HR75	C_SP	C_DP
Total	72	8.3 (0.5)	24.6 (1.3)	114 (1.5)	78.8 (0.9)
Men	18	4.1 (0.7)	12.3 (2.0)	114.1 (2.2)	79.3 (1.4)
Low PA	0	n/a	n/a	n/a	n/a
Mod PA	10	4.2 (1.0)	12.8 (2.5)	113.9 (3.1)	78.5 (1.7)
High PA	8	3.9 (1.2)	11.8 (3.3)	114.3 (3.1)	80.3 (2.4)
Women	54	9.8 (0.5)*	28.7 (1.2)*	114.5 (1.9)	78.6 (1.1)
Low PA	10	9.4 (1.1)	29.6 (2.4)	113.7 (3.7)	80.4 (1.8)
Mod PA	23	9.8 (0.8)	28.3 (1.6)	115.6 (3.5)	79.0 (1.8)
High PA	21	9.9 (0.9)	28.9 (2.2)	113.8 (2.7)	77.4 (1.7)

Data presented as mean (SE); C_AP_HR75, heart rate corrected central augmented pressure; C_AGPH_HR75, augmentation index; C_SP, central systolic pressure; C_DP, central diastolic pressure; *p<0.05 vs. Men.

Table 12. Central Hemodynamics at Different Physical Activity Levels

Group	N	C_AP_HR75	C_AGPH_HR75	C_SP	C_DP
Total	72	8.3 (0.5)	24.6 (1.3)	114 (1.5)	78.8 (0.9)
Low PA	10	9.4 (1.1)	29.6 (2.4)	113.7 (3.7)	80.4 (1.8)
Mod PA	33	8.1 (0.8)	23.6 (1.8)	115.1 (2.6)	78.8 (1.4)
High PA	29	8.2 (0.9)	24.1 (2.3)	113.9 (2.1)	78.2 (1.4)

Data presented as mean (SE); C_AP_HR75, heart rate corrected central augmented pressure; C_AGPH_HR75, augmentation index; C_SP, central systolic pressure; C_DP, central diastolic pressure.

Dietary and Supplemental Vitamin D Intake

Three-day average of dietary and supplemental vitamin D intakes for participants are presented in Table 13. Two way ANOVA detected no significant main effect (p<0.05) for gender for VITD IU (p=0.171) or VITD Supp (p=0.254) (Table 13). No significant main effect (p<0.05) for physical activity were detected for VitD IU (p=0.105) or VITD Supp (p=0.695) (Table 14). A Pearson correlation was conducted to ascertain if VITD IU (dietary vitamin D) was correlated with VIT D (circulating levels

of vitamin D). The correlation was low and not significant (r = 0.171; p=0.152). Gender (Males: r = 0.219; p=0.382, Females: r = 0.205; p=0.137) and physical activity level (low PA: r = 0.303; p=0.394, moderate PA: r = 0.052; p=0.772, high PA: r = 0.174; p=0.366) had minimal effect on these relationships.

Vitamin D is scarce in the diet and it is hard to meet the RDA (600 IU for ages 51-70) by diet alone, which is illustrated by the average intake of 89 IU in this study. Thus dietary vitamin D only explains about 3% of the variance seen in circulating (25(OH)D) vitamin D levels. A Pearson correlation was conducted to ascertain if VITD Supp (vitamin D supplement intake (IU)) was correlated with VIT D (circulating levels of vitamin D). The correlation was moderate (r = 0.544; p=0.000), explaining ~30% of the variance seen in the circulating vitamin D levels. Regarding gender, males (r = 0.745; p=0.000) had a strong correlation, explaining ~55% of the variance seen in the circulating vitamin D levels, while females (r = 0.522; p=0.000) had a moderate correlation, explaining ~27% of the variance seen in circulating vitamin D levels.

Table 13. Dietary & Supplemental Vitamin D

Group	N	Vit D (IU)	Vit D Supp (IU)
Total	72	89.0 (15.6)	1077.8 (201.1)
Men	18	126.2 (31.4)	677.8 (186.4)
Low PA	0	n/a	n/a
Mod PA	10	105.9 (28.3)	500.0 (227.5)
High PA	8	151.7 (62.6)	900.0 (306.5)
Women	54	76.6 (17.9)	1211.1 (259.2)
Low PA	10	26.3 (8.6)	1280.0 (975.8)
Mod PA	23	65.0 (20.0)	1504.4 (404.5)
High PA	21	113.2 (39.4)	857.1 (213.8)

Data presented as mean (SE); Vit D IU, dietary vitamin D in international units; Vit D Supp, supplemental vitamin D in international units.

Table 14. Dietary & Supplemental Vitamin D at Different Physical Activity Levels

Group	N	Vit D (IU)	Vit D Supp (IU)
Total	72	89.0 (15.6)	1077.8 (201.1)
Low PA	10	26.3 (8.6)	1280.0 (975.8)
Mod PA	33	77.4 (16.4)	1200.0 (299.1)
High PA	29	123.8 (32.9)	869.0 (173.6)

Data presented as mean (SE); Vit D IU, dietary vitamin D in international units; Vit D Supp, supplemental vitamin D in international units.

Sun Exposure

Two-way ANOVA detected no significant main effect for gender for SunExSc ($p=0.973$), TimeSun ($p=0.522$), or SkinExSc ($p=0.837$) (Table 15). A significant main effect ($p<0.05$) was detected for physical activity for SunExSc ($p=0.022$) and SkinExSc ($p=0.035$). LSD post-hoc analysis revealed a significant increase for SunExSc between low PA and high PA ($p=0.019$) and for SkinExSc between low PA and moderate PA ($p=0.036$), with higher scores with increased PA (Table 16). For many individuals, increasing physical activity means getting outside for exercise and games in order to increase your sun exposure. A Pearson correlation was conducted with VIT D (circulating levels) and the components of the sun exposure questionnaire: 1) Time Outdoors; 2) Amount of Skin Exposed; 3) Sun Exposure Score (sum of the daily products of Time Outdoors and Skin Exposed). The correlations were not significant for any of the measures: SunExSc: $r = 0.171$; $p=0.152$; TimeSun: $r = 0.046$; $p=0.701$; SkinExSc: $r = 0.165$; $p=0.165$. These results could be due to the time of year, which affects the ability to make vitamin D cutaneously from UVB radiation. Also, SPF use was not documented in this study, as this can drastically reduce (>90%) the ability to

cutaneously synthesize vitamin D. Many older individuals put on SPF before they step outside and have not been told about the benefits of sensible sun exposure.

Table 15. Sun Exposure

Group	N	SunExSc	TimeSun	SkinExSc
Total	72	19.4 (1.4)	9.1 (0.4)	13.5 (0.6)
Men	18	19.4 (2.3)	9.6 (0.7)	13.7 (1.3)
Low PA	0	n/a	n/a	n/a
Mod PA	10	15.9 (2.2)	8.2 (0.7)	12.7 (1.3)
High PA	8	23.9 (4.1)	11.3 (1.0)	15.0 (2.4)
Women	54	19.3 (1.7)	8.9 (0.6)	13.4 (0.7)
Low PA	10	10.2 (1.7)	7.0 (1.2)	9.6 (0.8)
Mod PA	23	21.7 (2.6)	9.1 (0.9)	15.0 (1.1)
High PA	21	21.1 (2.8)	9.5 (0.9)	13.5 (1.2)

Data presented as mean (SE); SunExSc, sun exposure score; TimeSun, Time in the sun; SkinExSc, skin exposure score.

Table 16. Sun Exposure at Different Physical Activity Levels

Group	N	SunExSc	TimeSun	SkinExSc
Total	72	19.4 (1.4)	9.1 (0.4)	13.5 (0.6)
Low PA	10	10.2 (1.7)*	7.0 (1.2)	9.6 (0.8)**
Mod PA	33	20.0 (2.0)	8.9 (0.6)	14.3 (0.9)
High PA	29	21.8 (2.3)	10.0 (0.7)	13.9 (1.0)

Data presented as mean (SE); SunExSc, sun exposure score; TimeSun, Time in the sun; SkinExSc, skin exposure score. *$p<0.05$ vs. high PA; **$p<0.05$ vs. moderate PA.

Cognitive Function

Prior to statistical analysis, a detailed examination of the data was conducted and data were screened to ensure validity and to look for outliers. For the 2-Choice Reaction Time (CH2) test, one subject had a single test rescored due to mouse button reversal. For the Simple Reaction Time (SRT) test, one subjects' score was deemed an extreme outlier for being >3SD from the mean, and this score was eliminated. Pearson correlation coefficients were calculated between the vitamin D level in the blood and the Throughput Scores on the cognitive tests. No significant correlations were found

for any of the cognitive tests: 1) SRT: r = 0.065; p=0.591, 2) CDS: r = -0.142; p=0.235, 3) PRO: r = 0.030; p=0.804, 4) MTH: r = 0.016; p=0.893, 5) M2S: r = -0.097; p=0.417, 6) CH2: r = -0.097; p=0.600, 7) CDD: r = 0.054; p=0.651 (Table 17).

A one-way ANOVA was conducted to evaluate potential differences across the three levels of vitamin D status (Deficient, Insufficient, Sufficient) for each Throughput score (Simple Reaction Time (SRT); Code Substitution Learning (CDS); Procedural Reaction Time (PRO); Mathematical Processing (MTH); Match to Sample (M2S); 2-Choice Reaction Time (CH2); Code Substitution Delayed (CDD)). No significant differences existed for each outcome variable across levels of vitamin D status. 1) SRT: p=0.394; 2) CDS: p= 0.860; 3) PRO: p=0.993; 4) MTH: p=0.837; 5) M2S: p=0.203; 6) CH2: p=0.796; 7) CDD: p=0.271 (Table 17).

Table 17. Throughput scores

	Total (25(OH)D)	Group 1 (Def)	Group 2 (Insuff)	Group 3 (Suff)	*p* (ANOVA)	r^a
N	72	9	27	36		
ANAM Tests, mean (std.)						
Simple Reaction Time	217.6 (262.8)	207.4 (33.7)	215.9 (27.6)	221.1 (24.5)	.394	.07
Code Sub Learning	37.3 (7.6)	36.9 (6.9)	38.0 (7.6)	37.0 (8.0)	.860	-.14
Proced React Time	95.7 (13.4)	95.8 (19.5)	96.0 (13.4)	95.6 (12.0)	.993	.03
Math Processing	25.1 (6.6)	25.8 (8.4)	24.5 (6.4)	25.4 (6.5)	.837	.02
Match to Sample	28.3 (8.0)	27.2 (8.4)	30.4 (7.3)	26.9 (8.3)	.203	-.10
2-Choice React Time	126.1 (15.3)	125.6 (15.1)	124.7 (16.2)	127.4 (15.1)	.796	-.10
Code Sub Delayed	34.2 (11.2)	31.0 (5.0)	36.8 (12.3)	33.0 (11.2)	.271	.05

[a]Pearson correlation between vitamin D level and ANAM Throughput score. Throughput is a hybrid measure of reaction time and accuracy. Reported as correct responses per minute. Higher values indicate better performance. P ANOVA, one-way ANOVA: Throughput scores across levels of vitamin D status (Deficient, Insufficient, Sufficient).

The Automated Neuropsychological Assessment Metrics (ANAM) Performance Report was utilized to compare to age norms. The ANAM Data Extraction & Prevention Tool (ADEPT) was utilized to classify subjects into one of three categories: 1) Average or above; 2) Below average (<9th %ile); 3) Clearly below average (<3rd %ile). 37.5% of subjects (27 out of 72) had at least 1 test below average or worse (<9th %ile), and 27.8% of subjects (20 out of 72) had at least 1 test clearly below average (<3rd %ile). A Chi-square analysis involving VITD Status and ANAM score

(Average=all 7 tests scored average; Below Average=at least 1 test scored below average) was conducted to examine the association and the sampling frequency between these two variables, and the association was not significant (p=0.766) (Table 18).

Table 18. Chi-Square Analysis

ANAM * VITD_Status Crosstabulation (p=0.766)					
Count					
		VITD_Status			Total
		Deficient	Insufficient	Sufficient	
ANAM Tests	Average	6	18	21	45
	Below Average	3	9	15	27
Total		9	27	36	72

Vitamin D Levels (25(OH)D)/Vitamin D Status

Pearson correlations were conducted to ascertain if circulating vitamin D levels (25(OH)D) was correlated with risk factors for chronic disease. The correlations were significant but weak as 25(OH)D was negatively correlated with GLU (r = -0.377; p=0.001), TG (r = -0.266; p=0.024), and A/G Ratio (r = -0.317; p=0.007). Thus, 25(OH)D only accounts for ~14% of the variance in fasting glucose levels, ~7% of the variance in triglyceride levels, and ~10% of the variance seen in A/G Ratio. Pearson correlations were also calculated to ascertain if 25(OH)D affects the risk for chronic disease differently for males and females. For males, correlations were significant and moderate as 25(OH)D was negatively correlated with P_SP (r = -0.557; p=0.016), P_MEANP (r = -0.496; p=0.036), C_SP (r = -0.534; p=0.022). Thus, 25(OH)D accounts for ~31% of the variance in P_SP, ~25% of the variance in P_MEANP, and ~29% of the variance in C_SP. For females, the correlations were significant but weak

as 25(OH)D was negatively correlated with GLU (r = -0.386; p=0.004), TG (r = -0.296; p=0.030), A/G Ratio (r = -0.425; p=0.001). Thus, 25(OH)D accounts for ~15% of the variance in GLU, ~9% of the variance in TG, and ~18% of the variance in A/G Ratio.

One-way ANOVA was conducted to evaluate potential differences across the three levels of vitamin D status (Deficient, Insufficient, Sufficient) for each risk factor for chronic disease. A significant main effect was detected for vitamin D status for GLU (p=0.001), TG (p=0.029), % BF (p=0.042), and A/G Ratio (p=0.007). Least Significant Difference (LSD) post-hoc analysis revealed a significant decrease for GLU between Deficient Vitamin D Status and Insufficient Vitamin D Status (p=0.002) and between Deficient Vitamin D Status and Sufficient Vitamin D Status (p=0.000), with GLU levels decreasing as vitamin D status improved; a significant decrease for TG between Deficient Vitamin D Status and Insufficient Vitamin D Status (p=0.024) and between Deficient Vitamin D Status and Sufficient Vitamin D Status (p=0.009), with TG levels decreasing with improved vitamin D status; a significant decrease for % BF between Deficient Vitamin D Status and Insufficient Vitamin D Status (p=0.012) and between Deficient Vitamin D Status and Sufficient Vitamin D Status (p=0.047), with % BF decreasing with improved vitamin D status; a significant decrease in A/G Ratio between Insufficient Vitamin D Status and Sufficient Vitamin D Status, with A/G Ratio decreasing with improved vitamin D status. Next, data was split based on gender and males had a significant difference for P_SP (p=0.016), P_MEANP (p=0.036), and C_SP (p=0.022), while females had a significant difference for GLU (p=0.003) and A/G Ratio (p=0.006). LSD post-hoc analysis revealed a significant decrease for GLU in females between Deficient Vitamin D Status and Insufficient Vitamin D Status (p=0.004) and

between Deficient Vitamin D Status and Sufficient Vitamin D Status (p=0.001), with GLU decreasing in females with improved vitamin D status; a significant decrease in A/G Ratio in females between Deficient Vitamin D Status and Sufficient Vitamin D Status, with A/G Ratio decreasing in females with improved vitamin D status. Post-hoc tests were not possible for males due to no males being in the Deficient Vitamin D Status group. All differences in the males were between Insufficient and Sufficient Vitamin D Status. Finally, data was split based on physical activity levels and the High Physical Activity Group had a significant difference for GLU (p=0.018) while Moderate Physical Activity Group had a significant difference for A/G Ratio (p=0.023). The Low Physical Activity Group had no significant differences for any of the risk factors. LSD post-hoc analysis revealed a significant decrease in GLU levels in the High Physical Activity Group between Deficient Vitamin D Status and Insufficient Vitamin D Status (p=0.044) and between Deficient Vitamin D Status and Sufficient Vitamin D Status (p=0.006). Post-hoc tests were not possible for Moderate Physical Activity Group due to one Vitamin D Status Group (Deficient) having only 1 subject, thus all differences in Moderate Physical Activity Group were between Insufficient and Sufficient Vitamin D Status.

A Chi-square analysis involving VITD_Status and VITD Synthesis was conducted to examine the association between these two variables. VITD Synthesis refers to the ability of an individual to synthesize vitamin D cutaneously via UVB radiation based on the time of year (1=March-October: Yes; 2=November-February: No). This analysis evaluated the association and the sampling frequency between the date (VITD SYN1 = March-October; VITD SYN2 = November-February) the

participant had their vitamin D measured with their vitamin D status. The association between the two variables was significant (p=0.036) and the results revealed that 31.3% (5/16) of the subjects tested between November to February were vitamin D deficient vs. only 7% (4/56) of those tested during March to October were vitamin D deficient. Also, 53.6% (30/56) of those tested March to October were vitamin D sufficient vs. only 37.5% (6/16) of those tested November to February were vitamin D sufficient. Another finding indicated that 83.3% (30/36) of participants with sufficient (>30 ng/ml) vitamin D status were tested between March to October vs. only 16.7% (6/36) of those tested between November to February. Finally, 62.5% of participants tested between November to February were not vitamin D sufficient (>30 ng/ml) (Table 19).

Table 19. Chi-Square Analysis

VITD_Status * VITD SYN Crosstabulation (p=0.036)					
			VITD SYN		Total
			YES	NO	
VITD_Status	Deficient	Count	4	5	9
		% within VITD_Status	44.4%	55.6%	100.0%
		% within VITD SYN	7.1%	31.3%	12.5%
		% of Total	5.6%	6.9%	12.5%
	Insufficient	Count	22	5	27
		% within VITD_Status	81.5%	18.5%	100.0%
		% within VITD SYN	39.3%	31.3%	37.5%
		% of Total	30.6%	6.9%	37.5%
	Sufficient	Count	30	6	36
		% within VITD_Status	83.3%	16.7%	100.0%
		% within VITD SYN	53.6%	37.5%	50.0%
		% of Total	41.7%	8.3%	50.0%
Total		Count	56	16	72
		% within VITD_Status	77.8%	22.2%	100.0%
		% within VITD SYN	100.0%	100.0%	100.0%
		% of Total	77.8%	22.2%	100%

Discussion

The primary objective of this study was to investigate any correlations between the current vitamin D status of the subjects and risk factors (components of metabolic syndrome and cognitive dysfunction) for chronic disease. The population in this study (males and females aged 50-70) is ideal to investigate for numerous reasons. Middle age to older adults in US society tend to accumulate body weight, add risk factors for chronic disease, and decrease physical activity. Deficient/insufficient vitamin D status is common due in part to this decreased physical activity (less sun exposure), decreased capacity of cutaneous synthesis of vitamin D when actually exposed to UVB radiation, and few foods naturally containing vitamin D. Many tissues and cells in the body have vitamin D receptors, thus research is ongoing and increasing to discover the link between vitamin D and many disease processes.

Main Findings

Circulating Vitamin D (25(OH)D) Levels/Vitamin D Status

The main contributors to vitamin D status include sensible sun exposure, vitamin D supplementation, and dietary intake of vitamin D, usually in this descending order. With older populations, sensible sun exposure is often minimal due to concerns over premature aging, decreased outdoor activities/exercise, and perceived skin cancer risk. What contributed or was associated with vitamin D status for the subjects in this study is very interesting, and it requires an individual to be aware of his/her habits regarding vitamin D all year long. The following will help describe what contributed to vitamin D status and vitamin D levels in this study.

In this study, exactly 50% (36 out of 72) of the study population was vitamin D deficient/insufficient (<30 ng/ml). 66.7% (12 out of 18) of the males were vitamin D deficient and/or insufficient vs. 44.4% (24 out of 54) of females, which is not unexpected due to a higher A/G Ratio and central obesity (70). This is not unusual, as vitamin D deficiency/insufficiency is common in US adults due in part to less time spent outdoors, increased use of sunscreen and protective clothing while outdoors, and vitamin D not widely available in the food supply (36, 44). These patterns were evident in this study population, as sensible sun exposure was minimal, explained by a mean sun exposure score of 19.4 (out of 56) which was equivalent to being outside most days of the week for only 10-15 minutes with minimal skin exposure (usually just hands and face, occasionally (1-2x/wk) exposing arms/legs). Next, dietary intake of vitamin D was very low (mean of 89 IU), as the RDA for vitamin D for this age group is 600 IU/d. The average vitamin D supplement intake for these subjects was quite substantial at 1078 IU, which moderately correlated with circulating vitamin D (25(OH)D levels ($r = 0.544$; $p=0.000$). This level of vitamin D supplementation is recommended by many vitamin D researchers in order to maintain a sufficient (>30ng/ml) vitamin D status (57, 63). The average circulating vitamin D (25(OH)D) level for all subjects was sufficient (>30ng/ml) at 31.1ng/ml. In addition, 86% of the study subjects self-reported at least a moderate physical activity level, and there was a main effect for physical activity ($p=0.014$). Average vitamin D levels were sufficient for the moderate and high physical activity groups and insufficient for the low physical activity group. In fact, 70% of the subjects in the low physical activity group had Deficient and/or Insufficient Vitamin D Status vs. 51.5% and 41.4% in the moderate and high physical activity groups,

respectively. Brock et al. (19) studied middle-aged men and women and also found that low vitamin D status is associated with physical inactivity, obesity, and low dietary vitamin D intake. Vitamin D status was associated with GLU, TGs, % BF, and A/G Ratio in this study, with GLU, TGs, % BF, and A/G Ratio all decreasing with improved vitamin D status. This is seen in the literature as low vitamin D status is associated with a wide variety of chronic disease risk factors (19, 25, 50, 69, 90, 100, 119).

There is a strong association between the time of year that you get your vitamin D levels measured and your vitamin D status. The crosstabulation analysis involving VITD_Status and VITD Synthesis really illustrates that it is more difficult to maintain sufficient vitamin D status during the winter months. The findings of this analysis that were reported in the Results section are common themes seen across the country and in the research literature (127, 129). It is very crucial that the public understand how diligent and consistent they must be with their dietary and supplemental vitamin D intake during the winter months in order to maintain sufficient (>30ng/ml) vitamin D status. Norman, OK is at 35 degrees latitude, and research has shown that no vitamin D can be produced in the skin from sun exposure from November-to-February when living above 33 degrees latitude (129). The solar zenith angle, which is the angle made by the sun's light (vs vertical) is increased at higher latitudes and during the winter months, so that UVB radiation has to travel farther distances and gets absorbed by the ozone layer so less UVB photons reach the earth's surface. Combine this with up to a twofold decline in the epidermal stores of the vitamin D precursor 7-DHC with aging, and this older study population is at risk for vitamin D deficiency, especially in the winter months (80, 120).

The importance of sensible sun exposure (without SPF, which reduces the capacity of the skin to make vitamin D by >95%) cannot be underestimated, as there are many advertisements, public service announcements, and even governmental programs that attempt to scare people away from the sun, and the general public is not exposed to the benefits of sensible sun exposure in the media. Even Healthy People 2020 has a goal of a 10% improvement in the number of adults who report that they are very likely to limit their sun exposure, use sunscreen, or wear protective clothing. In fact, the new RDAs for vitamin D were created for an individual receiving minimal sun exposure due to the perceived skin cancer risk and variability in cutaneous vitamin D synthesis via UVB radiation (102). The message of the benefits of sensible sun exposure is clearly not getting out to the public at large, and this leads to a large portion of our population missing out on the best and easiest method of maintaining sufficient vitamin D status.

If an individual gets regular sensible sun exposure (preferably through exercise), eats breakfast everyday (vitamin D fortified breakfast foods like cereal, milk, orange juice, and eggs), and adds a vitamin D supplement (especially in the winter), they will take in the recommended 1000-2000 IU/d that most vitamin D researchers recommend to reach sufficient vitamin D status so that they can take advantage of the potential extra skeletal benefits.

Body Composition and Vitamin D

Our results show that the females in the low PA group were the heaviest and had the highest BMI, %BF, A/G Ratio, WCcm, as well as the lowest circulating vitamin D (25(OH)D) levels. These results put these females in the obese category and at increased risk for chronic disease. The males in this study also had BMI, %BF, A/G

Ratio, and WCcm measures that put them in overweight/obese and at risk categorizations. The least physically active males had the highest BMI, %BF, and WCcm and also had the lowest circulating vitamin D (25(OH)D) levels. These results reinforce numerous studies where researchers found that overweight/obese individuals tend to have a worse vitamin D status than those with less adipose tissue (19, 68, 74, 90, 100, 132). The exact mechanism as to why overweight/obese individuals tend to have lower 25(OH)D levels is not completely understood. Vitamin D sequestration inside adipose tissue is a possible explanation. Worstman and Brock have both done studies where high BMI (>30) is significantly ($p<0.05$) associated with low 25(OH)D levels, with Worstman doing a UVB exposure study and the obese subjects had a 57% lower vitamin D_3 concentration than the non-obese subjects after UVB exposure (19, 132).

There are other hypotheses for lower vitamin D status in overweight/obese individuals, and one of them also could lead to a strategy to improve vitamin D status. Drincic et al. (32) used a volumetric dilution model to conclude that if you adjust for body weight/size in these overweight/obese individuals, there is no longer any difference between overweight/obese and healthy individuals in vitamin D status. They concluded that treatment based on body weight and a range of 70-80 IU/kg/day would be needed to produce sufficient vitamin D status in these overweight/obese individuals. Vitamin D intake based on body weight/size has been shown in numerous studies (4, 40, 41, 75, 77), and having this knowledge creates an easy and effective strategy for the overweight/obese population to eliminate their vitamin D deficiency/insufficiency and help create a healthier environment in their metabolically active adipose tissue.

In addition to knowing that one needs more vitamin D if they are overweight/obese, losing weight leads to improved 25(OH)D levels (99). Losing weight via outdoor exercise is the optimal scenario in this situation, as vitamin D status would be improved not only by the weight loss but also by the sun exposure. Adipose tissue expresses all of the vitamin D metabolizing enzymes (25-hydroxylase, 1α-hydroxylase (CYP27B1), 24-hydroxylase (catabolic CYP24A1)) and VDR. Decreased expression levels of these enzymes has been demonstrated in obese individuals. But after weight loss an increased expression of CYP24A1 was seen, which possibly means the active form $1,25(OH)_2D$ was created, exerted its positive effects, and then was degraded (121). Subjects in this study, who were overweight/obese, were also fairly physically active, so if this activity level could result in weight loss, they could see an improvement in their 25(OH)D levels and an increase in the expression of their vitamin D metabolizing enzymes, which can lead to a healthier inflammatory environment, as vitamin D has anti-inflammatory effects. This has potential implications for improving multiple components in cardiovascular disease prevention.

Lipid Panel and Glucose

Adipose tissue is a highly metabolic tissue that is involved in lipid and glucose metabolism. With the fact that subjects in this study were overweight/obese, this creates a chronic inflammatory state with adipose tissue dysfunction. A variety of hormones and cytokines are produced in adipose tissue, and overweight/obese individuals have an increased secretion of pro-inflammatory cytokines (tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) that can lead to increased inflammation, macrophage infiltration, and endothelial adhesion leading to increased risk of

atherosclerosis (50, 69, 119). Vitamin D has anti-inflammatory effects, as a 25(OH)D dose dependent downregulation of TNF-α and IL-6 has been shown as well as endothelial cell synthesis of $1,25(OH)_2D$ in response to inflammatory cytokines in vitro. Thus a sufficient vitamin D status would create an anti-inflammatory environment with the possibility of an autocrine response to combat atherosclerosis progression (133, 134). The subjects in this study had elevated LDL levels, as oxidized LDL initiates the cascade leading to inflammation, macrophage infiltration, and endothelial adhesion that leads to atherosclerosis. Total cholesterol was borderline high for the subjects in this study, but this situation was helped out by the high HDL levels seen. This elevated HDL level can potentially help the vitamin D status for these subjects, especially in the winter which we illustrated earlier is when subjects had the most difficulty maintaining sufficient vitamin D status. Cholesterol plays an important role in the barrier function of the skin, especially during the winter when the outside elements create more stress and more cholesterol is needed. Cholesterol is available either by endogenous synthesis (via 7-DHC) or from the plasma (HDL scavenging for cholesterol). If there is a reduction in cholesterol concentration for barrier function in the skin, then 7-DHC reductase activity (the enzyme that converts 7-DHC to cholesterol) increases. But with the optimal HDL concentration of the subjects, they will have an influx of cholesterol from its scavenging activity, thus allowing for increased 7-DHC concentration for increased vitamin D synthesis (94), provided you live below 33 degrees latitude. Exercise also increases HDL levels, which was illustrated both in the males and females in the study. Triglycerides, another cardiovascular risk factor associated with low

vitamin D status (82), also decreased with increased physical activity in the females of the study.

As someone ages, how well their blood glucose is controlled seems to influence overall health greatly and helps determine chronic disease risk. Overall, subjects who had normal fasting glucose levels, and lower glucose levels also had increased physical activity levels. Subjects also had increasing vitamin D levels that parallel decreasing glucose levels. This may be due to the fact that VDR are in pancreatic β-islet cells, and 1,25(OH)2D3 helps enhance insulin production and secretion (62, 124, 126). Numerous studies have illustrated the inverse relationship between 25(OH)D levels and fasting glucose levels (25, 42, 90). In addition to improved insulin secretion from β-cells, decreased insulin resistance at target tissues is also hypothesized. A vitamin D response element (VDRE) in the human insulin receptor promoter has been identified and is thought to contribute positively to insulin sensitivity (81). Beydoun et al. (14) saw a stronger inverse association of 25(OH)D with fasting glucose among subjects with central adiposity vs. those without central adiposity. Both men and women in this study had A/G Ratio and waist circumference values that put them at increased risk for chronic disease as well as lower 25(OH)D levels. With increased central adiposity there is also an increased risk of developing non-alcoholic fatty liver disease (NAFLD). 25(OH)D is produced in the liver, thus excess adipose tissue, especially central adiposity, can negatively affect circulating 25(OH)D levels.

Dietary Intake

When taking into account the body composition, glucose, and lipid panel values of the subjects, it becomes apparent that improved dietary practices could improve these

outcome variables and decrease risk factors. Percent of kcals from fat was at the upper end of the Acceptable Macronutrient Distribution Range (AMDR) for fat. If subjects decreased their fat intake and increased their fiber intake, it would have a positive effect on their body fat levels and also their lipid levels, as dietary fiber decreases cholesterol levels in the body. All subjects were low in their fiber intake and did not meet recommended levels (M: 38g/d; F: 25g/d).

Hemodynamics

Blood pressure, peripheral and central, were at normal values for this study population. With aortic pressure prominent in the heart, kidneys, and brain, many researchers believe that central pressure is related much stronger to cardiovascular events than brachial pressure (86). With the overweight/obese situation of these study subjects, they are definitely at increased risk for hypertension. This study did not control for blood pressure medication, and 25% of the subjects were taking some form of hypertensive medication. Since the mean circulating vitamin D (25(OH)D levels for the study subjects was 31.1 ng/ml, those with sufficient vitamin D status could potentially garner benefits with better blood pressure control. This is due to the fact that evidence exists that $1,25(OH)_2D_3$ regulates the major blood pressure regulating hormone renin in the kidneys. The active vitamin D hormone functions as an inhibitor of the renin-angiotensin system (RAS), which if over activated leads to hypertension. The hypothesized mechanism is a negative regulation of renin gene transcription via a VDR-mediated mechanism and/or VDR and 1α-hydroxylase activity on blood vessel walls (79, 108, 112). Vitamin D binding protein (DBP), a novel growth factor, is released during endothelial stress. If endothelial stress is consistently excessive, as in

hypertension, the repair process can become flawed and increase your risk for atherosclerosis and cardiovascular events. DBP is released and leads to the migration of vascular smooth muscle cells (VSMCs) to sites of endothelial damage, which could occur in this study population due to their elevated LDL levels. DBP only signals the VSMCs to proliferate and migrate if 25(OH)D and $1,25(OH)_2D_3$ are not bound to its binding site. Phosphate and TNF-α (both increased in low vitamin D status) increase osteogenic processes in VSMCs and may increase the risk for vascular calcification. Thus sufficient vitamin D status may play a protective role in maintaining vascular health (22, 98, 114). The females in this study had elevated measures of arterial stiffness (C_AP_HR75, C_AGPH_HR75) compared to the males. The females were the only gender that made up the low physical activity group, and the low physical activity group also had the lowest 25(OH)D levels. The inability to properly protect and maintain vascular health due to low vitamin D levels is a potential explanation as to why females had higher arterial stiffness values.

Cognitive Function

With vascular health being such a vital component of optimal cognitive functioning of the brain, it is easy to see how Alzheimer's disease (AD) and dementia are major issues, with the cognitive dysfunction occurring secondarily to the cerebrovascular and cardiovascular disease seen in society today. While 37.5% of subjects (27 out of 72) had at least 1 test below average or worse ($<9^{th}$ %ile), and 27.8% of subjects (20 out of 72) had at least 1 test clearly below average ($<3^{rd}$ %ile), a chi-square analysis involving vitamin D status and ANAM scores (Average = all 7 tests scored average; Below Average = at least 1 test scored below average) was conducted

and the association between the two variables was not significant (p=0.766). Our results regarding vitamin D and these tests of executive function (attention, processing speed, working memory, spatial processing) did not show an association, but Annweiler et al. (10) found that lower circulating vitamin D levels predicted executive dysfunction. Annweiler et al. (6) also concluded that <10 ng/ml seems to be the threshold that is most associated with cognition, as this would signify chronic hypovitaminosis D and has probably contributed to brain dysfunction over an extended period of time.

When you combine the obesity, elevated LDL levels, and high dietary fat intake of these study subjects, they are at increased risk for cognitive dysfunction later in life. Gorelick et al. (48) highlighted the importance of controlling your blood pressure in mid-life to prevent dementia rather than wait until you are elderly. Being proactive and trying to reduce or eliminate all risk factors for chronic disease will also help prevent cognitive dysfunction later in life. Annweiler et al. (7) and other experts recently gathered to discuss important points regarding vitamin D and cognition in older adults. They concluded that hypovitaminosis D (< 30 ng/ml) is a risk factor for cognitive disorders and that vitamin D supplementation should be implemented early in the care process to ensure vitamin D sufficiency (> 30ng/ml). While vitamin D status by itself is not specific enough to diagnose cognitive disorders, it is part of the equation and with many biological targets throughout the body, vitamin D levels most likely contribute to the wide variety of symptoms seen in older adults with cognitive disorders.

In addition to the anti-inflammatory and vascular maintenance properties of vitamin D that have already been mentioned, $1,25(OH)_2D_3$ is being investigated for its role in stimulating amyloid-beta, the major protein that accumulates and effects neuron

function, phagocytosis and clearance (83). Vitamin D is being combined with AD medication to see if it is more effective than the drug or vitamin D alone (8). Vitamin D was combined with the AD drug Memantine and given to newly diagnosed AD patients for 6 months, and cognitive change was assessed with the Mini-Mental State Examination (MMSE), which is what was used in this study. At 6 months, the memantine plus vitamin D group increased their MMSE score by 4.0 points and had a statistically and clinically relevant gain in cognition. So vitamin D could possibly be part of the AD solution in a big way in the future. With an aging population, vitamin D may play a role in maintaining cognitive function and be part of effective intervention to help delay cognitive dysfunction.

CHAPTER V

CONCLUSIONS

Purpose

The purpose of the present study was to investigate the relationship between current vitamin D status and risk factors (components of metabolic syndrome and cognitive dysfunction) for numerous chronic diseases (cardiovascular disease, diabetes, dementia) in men and women aged 50-70 years. This study aimed to determine if deficient and/or insufficient vitamin D status was associated with risk factors (components of metabolic syndrome and cognitive dysfunction) for chronic disease (cardiovascular disease, diabetes, cognitive dysfunction).

Hypotheses

1. The vitamin D status of at least 50% of these older individuals will be deficient and/or insufficient.

Yes, the results of the current study support this hypothesis. Exactly 50% of the study population (36 out of 72) were vitamin D deficient (9) or insufficient (27).

2. Deficient and/or insufficient vitamin D status will be associated with chronic disease risk factors.

Yes, the results of the current study support this hypothesis. Deficient and/or insufficient vitamin D status was associated with GLU, TGs, % BF, and A/G Ratio.

3. Circulating vitamin D levels (25(OH)D) will correlate with risk factors for chronic disease and males will have stronger correlations with risk factors for chronic disease vs. females.

Yes, the results of the current study support this hypothesis. For males the correlations were moderate as vitamin D levels were negatively correlated with P_SP (r = -0.557; p=0.016), P_MEANP (r = -0.496; p=0.036); C_SP (r = -0.534; p=0.022). Thus, vitamin D levels account for ~31% of the variance in P_SP, ~25% of the variance in P_MEANP, and ~29% of the variance in C_SP. For females, the correlations were significant but weak as vitamin D levels were negatively correlated with GLU (r = -0.386; p=0.004), TG (r = -0.296; p=0.030), A/G Ratio (r = -0.425; p=0.001). Thus, vitamin D levels account for ~15% of the variance in GLU, ~9% of the variance in TG, and ~18% of the variance in A/G Ratio.

4. Dietary intake of vitamin D will not differ based on gender or physical activity level.

Yes, the results of the current study support this hypothesis. No significant main effect for gender for dietary vitamin D (p=0.171) or for physical activity for dietary vitamin D (p=0.105) was detected.

5. Supplemental intake of vitamin D will not differ based on gender or physical activity level.

Yes, the results of the current study support this hypothesis. No significant main effect for gender for supplemental vitamin D (p=0.254) or for physical activity for supplemental vitamin D (p=0.695) was detected.

6. Correlations between dietary intake of vitamin D and circulating levels of vitamin D (25(OH)D) will not differ based on gender or physical activity level.

Yes, the results of the current study support this hypothesis. The correlation was low and not significant ($r = 0.171$; $p=0.152$) and gender and physical activity had minimal effect on these relationships.

7. Circulating vitamin D levels (25(OH)D) will not correlate with Throughput scores on cognitive tests.

Yes, the results of the current study support this hypothesis. All the correlations were low and not significant.

Subquestion Hypotheses

1. A) The prevalence of deficient and/or insufficient vitamin D status will be greater in males vs. females.

Yes, the results of the current study support this hypothesis. 66.7% (12 out of 18) of the males were vitamin D deficient and/or insufficient vs. 44.4% (24 out of 54) of the females were vitamin D deficient and/or insufficient.

B) The prevalence of deficient and/or insufficient vitamin D status will be greater in the low physical activity group vs. the moderate or high physical activity group.

Yes, the results of the current study support this hypothesis. 70% (7 out of 10) of the subjects in the low physical activity group were vitamin D deficient and/or insufficient vs. 51.5% (17 out of 33) and 41.4% (12 out of 29) for the moderate and high physical activity groups respectively.

2. A) Males with a deficient and/or insufficient vitamin D status will be associated with more risk factors for chronic disease vs. females.

Yes, the results of the current study support this hypothesis. Males with a deficient and/or insufficient vitamin D status were associated with 3 risk factors (P_SP, P_MEANP, C_SP) vs. 2 risk factors (GLU, A/G Ratio) for females.

B) Subjects in the low physical activity group with a deficient and/or insufficient vitamin D status will not see a significant difference in risk factors for chronic disease (across levels of vitamin D status) vs. subjects in the moderate and high physical activity groups with a deficient and/or insufficient vitamin D status.

Yes, the results of the current study support this hypothesis. No significant difference for any risk factor was seen in the low physical activity group between the different levels of Vitamin D Status, while a significant decrease in GLU levels was seen in the high physical activity group between Deficient Vitamin D Status and Sufficient Vitamin D Status ($p=0.006$). In the moderate physical activity group a significant difference was seen for A/G Ratio between Insufficient Vitamin D Status and Sufficient Vitamin D Status ($p=0.023$).

Strengths and Limitations

The results of this study are limited due to the cross-sectional design, thus you cannot draw cause-and-effect relationships with the participants' vitamin D levels/status and their risk factors for chronic disease. In addition, exclusion criteria did not include high blood pressure or cholesterol medication. Recruitment was spread out over a 10 month period, so when vitamin D status was assessed for 16 subjects, it was not possible to synthesize vitamin D cutaneously via UVB radiation because the sun angle was too low. Next, the sun exposure questionnaire did not collect information

regarding sunscreen use or type of clothing worn. In addition, there may not have been enough practice on the ANAM cognitive tests for sufficient familiarity and optimal performance, as the practice test was an abbreviated 5 minute version that was given only once, usually a week or more in advance of the actual test. Finally, the low number of subjects (72) and gender discrepancy (54 females, 18 males) could affect variances and ANOVA results seen, and may result in a greater chance of a Type II error, or the failure to detect an effect that might be present.

This study's strengths included a direct measure of circulating vitamin D levels, a 3-day food record, a vitamin D food/supplementation frequency questionnaire, and a sun exposure questionnaire in order to accurately assess vitamin D status and determine what components most contribute to one's vitamin D status.

Significance

This study confirmed that a deficient/insufficient vitamin D status is common in an older population and is associated with increased risk factors for chronic disease. Having physicians routinely assess vitamin D status in their patients, as part of an annual physical exam, just as they do for a lipid panel or fasting glucose, seems a logical next step. In addition, there are several factors that are associated with an increased risk of developing vitamin D deficiency. Physicians need to know that at risk populations include: 1) older adults, as the skin becomes less efficient at producing vitamin D with age due to diminished 7-DHC levels; 2) individuals with non-alcoholic fatty liver disease, which will continue to increase in the coming years due to the obesity epidemic. 25(OH)D synthesis occurs in the liver and this process can be compromised with a fatty liver; 3) individuals who live at higher latitudes or who spend

little time outside; 4) races with high melanin levels (African Americans, Native Americans, and Latinos) are at increased risk due to a reduced efficiency of vitamin D conversion in the skin. Vitamin D deficiency/insufficiency will continue to be an issue in the future, especially with the obesity epidemic still on the rise. Physicians need to begin to recommend sensible sun exposure to their patients, and incorporating that message when recommending increased physical activity would be valuable to the general public. Once vitamin D deficiency/insufficiency is diagnosed, it can be remedied rather cheaply and effectively with sun exposure, supplementation, and dietary recommendations.

Future Research

Future studies aimed at expanding on our findings need to include quality randomized controlled trials. There is now plenty of epidemiological data out that supports potential links between low vitamin D levels and increased risk for chronic disease. What is lacking are quality randomized controlled trials utilizing vitamin D supplementation. 25(OH)D can clearly be used as a biomarker for vitamin D status, but whether it can be a biomarker for certain health related outcomes has not been established and is still to be determined. With the obesity epidemic there are ample opportunities for vitamin D supplementation in weight loss and exercise intervention studies. Whether vitamin D can alter the incidence of chronic disease or its risk factors in vitamin D deficient/insufficient individuals has not been answered satisfactorily yet, and uncovering these mechanisms in the years and decades that follow will be intriguing.

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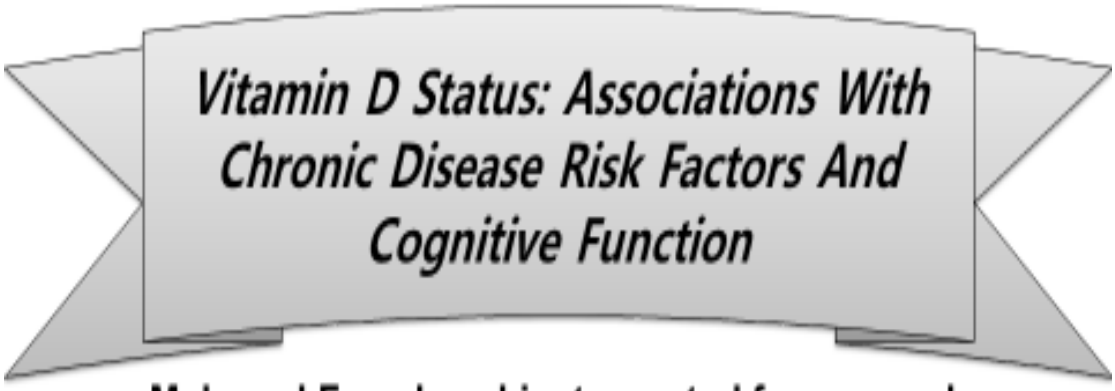
APPENDICES

Appendix A

Recruitment Materials



Male and Female subjects wanted for research

The University of Oklahoma is an Equal Opportunity Institution

To participate:
- 50 - 70 years of age
- less than 300 lbs.

-Receive FREE BONE DENSITY Results
-Receive FREE BODY FAT Results
-Receive FREE CHOLESTEROL Results

Requirements:
-One initial visit (about 60 min) to fill out questionnaires
-One testing session (about 90-120 min) for total body DXA scan (you will be exposed to a small amount of radiation from the DXA scan), computer test to assess cognitive function, and pulse wave measurement (similar to a blood pressure measure)
-One fasting blood draw (~15ml) visit

You will receive your research results at the end of the study upon your request!

Vitamin D Study
-Steve-
480-258-7302

E-mail Message

I invite you to participate in a research study, titled "Vitamin D Status: Associations With Chronic Disease Risk Factors and Cognitive Function."

The objective and purpose of this study is to investigate the relationship between current vitamin D status and risk factors (hypertension, metabolic syndrome, cognitive function) for numerous chronic diseases (cardiovascular disease, diabetes, dementia) in men and women aged 50-70 years. This study aims to determine if deficient and/or insufficient vitamin D status is associated with risk factors for chronic disease.

The total commitment for this study is as follows: 1) An initial visit (about 60 minutes) to fill out consent forms and questionnaires; 2) One visit to OU Health Services (Goddard Health Center) for 1 blood draw (~15 minutes); 3) One testing session visit (~90 minutes) – height, weight, waist circumference, blood pressure, pulse wave analysis, DXA scan (body composition), and cognitive function computer generated assessment tests.

This study requires that participants be fasting for the one blood draw. Participants will be exposed to a small amount of radiation due to the DXA scan

Please let me know if you might be interested in this study and if there are any questions I might be able to answer for you.

The University of Oklahoma is an Equal Opportunity Institution. Thank you for your interest.

Steven L. Ferguson MS, RD, CSCS

Steven.L.Ferguson-1@ou.edu

The OU IRB has approved the content of this message but not the method of distribution. The OU IRB has no authority to approve distribution by mass email.

Appendix B

IRB Approval Letter

The UNIVERSITY of OKLAHOMA

Institutional Review Board for the Protection of Human Subjects
Approval of Initial Submission – Board Review – AP01

Date: November 15, 2012

Principal Investigator: Michael G Bemben, Ph.D.

IRB#: 1568
Study Title: Vitamin D status: Associations with chronic disease risk factors and cognitive function

IRB Meeting Date: 11/13/2012

IRB Approval Date: 11/15/2012
IRB Expiration Date: 10/31/2013

Collection/Use of PHI: Yes

The review and approval of this submission is based on the determination that the study will be conducted in a manner consistent with the requirements of 45 CFR 46.

To view the approved documents for this submission, open this study from the My Studies option, go to Submission History, go to Completed Submissions tab and then click the Details icon.

You will receive notification approximately 60 days prior to the expiration date noted above. You are responsible for submitting continuing review documents in a timely fashion in order to maintain continued IRB approval.

You are also responsible for:

- Ensuring this research is conducted as approved by the IRB.
- Obtaining consent using the currently approved, stamped consent form and retaining all original, signed consent forms, if applicable.
- Informing the IRB of any/all modifications prior to implementing those changes.
- Reporting any serious, unanticipated harms as per Policy 407 and/or any additional information that may change the risk, benefit, or desire for participants to continue in the study.
- Submitting a final closure report at the completion of the project.
- Keeping and maintaining accurate study records as your study is subject to quality improvement evaluation.

If you have questions about this notification or using iRIS, contact the IRB @ 405-325-8110 or irb@ou.edu.

Cordially,

E. Laurette Taylor

E. Laurette Taylor, Ph.D.

Chair, Institutional Review Board

The UNIVERSITY of OKLAHOMA

Institutional Review Board for the Protection of Human Subjects

Continuing Review – Board Approval

Date: September 19, 2013

To: Michael G Bemben, PHD

IRB#: 1568
Meeting Date: 09/16/2013
Approval Date: 09/16/2013
Expiration Date: 08/31/2014

Study Title: Vitamin D status: Associations with chronic disease risk factors and cognitive function

Study Status: Active – Open

Reference Number: 566602

At its regularly scheduled meeting, the Institutional Review Board (IRB) reviewed the Application for Continuing Review for the above-referenced research study. Study documents (e.g. protocol, consent, survey, etc.) associated with this submission are listed on page 2 of this letter. To review or access the submission documents (e.g. application, review response form) as well as the study documents approved for this submission, open this study from the *My Studies* option, go to *Protocol Items*, click to open *Application, Informed Consent,* or *Other Study Documents* to find currently approved items.

As principal investigator of this research study, it is your responsibility to:

- Conduct the research study in a manner consistent with the requirements of the IRB and federal regulations at 45 CFR 46 and/or 21 CFR 50 and 56.
- Obtain informed consent and research privacy authorization using the currently approved, stamped forms and retain all original, signed forms, if applicable.
- Request approval from the IRB prior to implementing any/all modifications.
- Promptly report to the IRB any harm experienced by a participant that is both unanticipated and related per IRB Policy.
- Maintain accurate and complete study records for evaluation by the HRPP quality improvement program and if applicable, inspection by regulatory agencies and/or the study sponsor.
- Promptly submit continuing review documents to the IRB upon notification approximately 60 days prior to the expiration date indicated above.
- Submit a final closure report at the completion of the project.

If you have questions about this notification or using iRIS, contact the IRB @ 405-271-2045 or irb@ouhsc.edu.

Sincerely,

Karen Beckman, MD
Chairperson, Institutional Review Board

Appendix C

Informed Consent and Authorization to Use or Disclose Protected Health Information

University of Oklahoma
Institutional Review Board
Informed Consent to Participate in a Research Study

Project Title: Vitamin D Status: Associations with Chronic Disease Risk Factors and Cognitive Function
Principal Investigator: Michael G. Bemben, PhD
Department: Health and Exercise Science

You are being asked to volunteer for this research study. This study is being conducted at the University of Oklahoma in the Department of Health and Exercise Science. You were selected as a possible participant because you are 50-70 years old and weigh less than 300 lbs.

Please read this form and ask any questions that you may have before agreeing to take part in this study.

Purpose of the Research Study

The purpose of this study is to investigate the relationship between current vitamin D status and risk factors (hypertension, metabolic syndrome, cognitive dysfunction) for numerous chronic diseases (cardiovascular disease, diabetes, dementia) in men and women aged 50-70 years. This study aims to determine if deficient and/or insufficient vitamin D status is associated with risk factors (hypertension, metabolic syndrome, cognitive dysfunction) for chronic disease (cardiovascular disease, diabetes, dementia).

Number of Participants

Up to 100 males and postmenopausal females aged 50-70 years will take part in this study.

Procedures

The study will then require 3 visits to the Neuromuscular (Rm 6 HHC) & Bone Density (Rm 4 HHC) Research Laboratories for measurements that are linked to vitamin D status (serum Vitamin D, bone density) or to the risk factors mentioned above. Specifically, blood pressure and stiffness of the arteries are related to hypertension; serum lipids, blood glucose, body size and composition are related to the metabolic syndrome, and reaction time and accuracy are related to cognitive function.

If you agree to be in this study, you will be asked to:

1. Sign and date an informed consent document (this document) indicating that you understand all procedures and your rights as a research subject. You will then complete a HIPPA form, a Health Status Questionnaire, a Mini-Mental State Examination (MMSE), and an International Physical Activity Questionnaire. In addition, you will be asked to take home and complete a Sun Exposure Questionnaire, a 3-day food record, and a Vitamin D Intake Questionnaire. Finally, a familiarization session will be given on the cognitive function computer generated assessment tests. (Visit 1: about 60 minutes)
2. In the second visit, testing will be conducted for height, weight, waist circumference, blood pressure, arterial stiffness (pulse wave analysis), a total body DXA scan for body

composition, and cognitive function computer generated assessment tests. (Visit 2: about 90 minutes)

A. Body weight and height will be measured at the start of the test session. (about 2 minutes)
B. Waist circumference will be measured using a tape measure. (about 1 minute)
C. Blood pressure will be taken sitting down from the upper left arm (about 5-10 minutes)
D. Pulse Wave Analysis will be measured using a small, pen-shaped device that is connected to a machine called a SphygmoCor. You will lie down on a medical table for this test. After finding your pulse at the wrist with the pen-shaped device, pulse waves will be analyzed by the machine to obtain measures of blood pressure and arterial stiffness. (about 10 minutes)
E. Dual Energy X-ray Absorptiometry (DXA): A total body DXA scan will be performed to assess body composition and bone density. The subject will lie down on the DXA table, hands will be placed by the side of the legs, and Velcro straps will be placed around the ankles so that the participant will not have to hold his/her feet together for the duration of the scan. (about 10 minutes)
F. Cognitive Function: A specific battery of computer aided tests will be given in a specific order to measure cognitive function. (about 30 minutes)

3. One separate visit to OU Health Services (Goddard Health Center) will also be required for one blood draw. The blood draw must be done in the morning after an 8 hour overnight fast. The phlebotomist will fill 1-2 tubes (about 7.5 ml each) of blood from one venipuncture. These blood samples will be analyzed for a lipid panel (total cholesterol, HDL, LDL, VLDL, and ratios), as well as blood glucose and vitamin D concentrations. Blood samples will be kept for at least 2 years in case samples have to be reanalyzed. Also, it may be about 1 year before these blood results could be made available to you (upon your request). You will be contacted by a member of the research team if an abnormal test result is observed and encouraged to contact your personal physician. (Visit 3: about 15 minutes)

Length of Participation

1. Visit 1: Informed consent, questionnaires, and familiarization session. (about 60 minutes)
2. Visit 2: Will consist of measuring height, weight, waist circumference, blood pressure, pulse wave analysis, a total body DXA scan, and cognitive function computer generated assessment tests. (about 90 minutes)
3. Visit 3: A visit to OU Health Services (Goddard Health Center) will also be required for one fasting blood draw. This blood sample will be used to analyze blood lipids, blood glucose, and vitamin D status. (about 15 minutes)

Risks of being in the study are

1. Blood draws will be performed by qualified personnel, but there may be possible discomfort at the site of venipuncture and possible bruising during and after your blood draws.
2. This research study involves exposure to radiation from 1 DXA scan, which is a type of X-ray procedure. This radiation is not necessary for medical care, and is for research purposes only. The subject will receive radiation exposure of less than 2 mrem from the scan, which is less than the radiation received in 3 days from natural background

radiation (~300 mrem/year), such as naturally occurring radioactivity in the soil. Any risk from the amount of radiation is too small to measure directly, and is small when compared to everyday risks. Although the amount of radiation exposure received in the study is minimal, it is important for the subject to be aware that the risk from radiation exposure is cumulative over a lifetime. Any risk from the amount of radiation is too small to measure directly, and is small when compared to everyday risks.

Benefits of being in the study are

There are no direct benefits

Compensation

You will not be reimbursed for your time and participation in this study.

Injury

In case of injury or illness resulting from this study, emergency medical treatment is available. However, you or your insurance company will be expected to pay the usual charge from this treatment. The University of Oklahoma Norman Campus has set aside no funds to compensate you in the event of injury.

Confidentiality

In published reports, there will be no information included that will make it possible to identify you. Research records will be stored securely and only approved researchers will have access to the records.

There are organizations that may inspect and/or copy your research records for quality assurance and data analysis. These organizations include the OU Institutional Review Board.

Following collection, your blood sample will not be associated with any information that would identify you as the donor of this sample (i.e., it will be de-identified) and subsequently no attempt will be made to make that association. It is possible for your identity to be determined from this sample, but the chances of that occurring are highly unlikely.

Voluntary Nature of the Study

Participation in this study is voluntary. If you withdraw or decline participation, you will not be penalized or lose benefits or services unrelated to the study. If you decide to participate, you may decline to answer any question and may choose to withdraw at any time.

You have the right to access the research data that has been collected about you as a part of this research study. However, you may not have access to this information until the entire research study has completely finished and you consent to this temporary restriction.

Contacts and Questions

If you have concerns or complaints about the research, the researcher(s) conducting this study can be contacted at

Steven L. Ferguson (480)258-7302 Steven.L.Ferguson-1@ou.edu

Michael Bemben (405)325-2717 mgbemben@ou.edu

Contact the researcher(s) if you have questions, or if you have experienced a research-related injury.

If you have any questions about your rights as a research participant, concerns, or complaints about the research and wish to talk to someone other than individuals on the research team or if you cannot reach the research team, you may contact the University of Oklahoma – Norman Campus Institutional Review Board (OU-NC IRB) at 405-325-8110 or irb@ou.edu.

You will be given a copy of this information to keep for your records. If you are not given a copy of this consent form, please request one.

Statement of Consent

I have read the above information. I have asked questions and have received satisfactory answers. I consent to participate in the study.

Participant Signature Print Name Date

Signature of Person Obtaining Consent Date

Print Name of Person Obtaining Consent

UNIVERSITY OF OKLAHOMA – NORMAN CAMPUS
INSTITUTIONAL REVIEW BOARD

AUTHORIZATION TO USE or DISCLOSE PROTECTED HEALTH INFORMATION FOR RESEARCH

An additional Informed Consent Document for Research Participation may also be required.

Title or Research Project: Vitamin D Status: Associations With Chronic Disease Risk Factors and Cognitive Function

Principal Investigator: Michael G. Bemben

IRB Number: 1568

Address: 1401 Asp Ave. 115 HHC, Norman OK 73019

Phone Number: 405-325-2717

If you decide to join this research project, University of Oklahoma (OU) researchers may use or share (disclose) information about you that is considered to be protected health information for their research. Protected health information will be called private information in this Authorization.

Private information To be Used or Shared. Federal law requires that researchers get your permission (authorization) to use or share your private information. If you give permission, the researchers may use or share with the people identified in this Authorization any private information related to this research from your medical records and from any test results. Information, used or shared, may include all information relating to any tests, procedures, surveys, or interviews as outlined in the consent form, medical records and charts, name, address, telephone number, date of birth, race, and government-issued identification number.

Purposes for Using or Sharing Private Information. If you give permission, the researchers may use your private information to analyze the data from the project and present the information.

Other Use and Sharing of Private Information. If you give permission, the researchers may also use your private information to develop new procedures or commercial products. They may share your private information with the research

sponsor, the OU Institutional Review Board, auditors and inspectors who check the research, and government agencies such as the Department of Health and Human Services (HHS). The researchers may also share your private information with all researchers collaborating on this project.

Confidentiality. Although the research may report their findings in scientific journals or meetings, they will not identify you in their reports. The researchers will try to keep your information confidential, but confidentiality is not guaranteed. Any person or organization receiving the information based on this authorization could re-release the information to others and federal law would no longer protect it.

YOU MUST UNDERSTAND THAT YOUR PROTECTED HEALTH INFORMATION MAY INCLUDE INFORMATION REGARDING ANY CONDITIONS CONSIDERED AS A COMMUNICABLE OR VENEREAL DISEASE WHICH MAY INCLUDE, BUT ARE NOT LIMITED TO, DISEASES SUCH AS HEPATITIS, SYPHILIS, GONORRHEA, AND HUMAN IMMUNODEFICIENCY VIRUS ALSO KNOWN AS ACQUIRED IMMUNE DEFICIENCY SYNDROME (AIDS).

Voluntary Choice. The choice to give OU researchers permission to use or share your private information for their research is voluntary. It is completely up to you. No one can force you to give permission. However, you must give permission for OU researchers to use or share your private health information if you want to participate in the research and if you revoke your authorization, you can no longer participate in this study.

Refusing to give permission will not affect your ability to get routine treatment or health care from OU.

Revoking Permission. If you give OU researchers permission to use or share your private information, you have a right to revoke your permission whenever you want. However, revoking your permission will not apply to information that the researchers have already used, relied on, or shared.

End of Permission. Unless you revoke it, permission for OU researchers to use or share your private information for their research will end when all data from the project has been analyzed and all reports have been published. You may revoke your permission at any time by writing to:

Privacy Official
University of Oklahoma
1000 Stanton L. Young Blvd., STE 221,
Oklahoma City, OK 73117
If you have questions, call: (405) 271-2511

Giving Permission. By signing this form, you give OU and OU's researchers led by Michael G. Bemben, PhD., permission to share your private information for the research project called Vitamin D Status: Associations With Chronic Disease Risk Factors and Cognitive Function.

Subject Name:

_____ _____

Signature of Subject Date
Or parent if Subject is a Child

Or

_____ _____

Signature of Legal Representative** Date

**If signed by a legal Representative of the Subject, provide a description of the relationship to the Subject and the Authority to Act as Legal Representative:

_____

OU may ask you to produce evidence of your relationship.

A signed copy of this form must be given to the Subject or the Legal Representative at the time this signed form is provided to the researcher or his representative.

Appendix D

Study Questionnaires

Pre-Screening Subjects Recruitment Form

University of Oklahoma Neuromuscular Laboratory

"Vitamin D Status: Associations with Chronic Disease Risk Factors and Cognitive Function"

NAME: _____ DATE: _____ PHONE NUMBER: _____

Inclusion Criteria- The inclusion criteria for this study requires that each subject:

	YES	NO
Is a male or female aged 50-70	____	____
Female Subject: Postmenopausal	____	____
Is less than 300 lbs	____	____
Have no cognitive problems that could interfere with their participation	____	____

Exclusion Criteria-The exclusion criteria for this study require that each subject:

Is outside the age range	____	____
Female Subject: Not Postmenopausal	____	____
Is more than 300 lbs	____	____
Have cognitive impairment that does not allow them to complete the current process or testing	____	____
Qualified for study	____	____

PI Signature _____ Date _____

Bone Density Research Laboratory

OU Department of Health and Exercise Science

Health Status Questionnaire

Instructions Complete each question accurately. All information provided is confidential.

Part 1. Information about the individual

1._____
Date

2. _____
Legal name Nickname

3._____
Mailing address

_____
Home phone Business phone

4.Gender (circle one): Female Male

5. Year of birth: _____ Age: _____

6. Number of hours worked per week: Less than 20 20-40 41-60 Over 60

More than 25% of time spent on job (circle all that apply)

Sitting at desk Lifting or carrying loads Standing Walking Driving

Part 2. Medical history

7. Circle any who died of heart attack before age 50:

Father Mother Brother Sister Grandparent

8.Date of: Last medical physical exam: _____Last physical fitness test: _____
Year Year

9. Circle operations you have had:

Back	Heart	Kidney	Eyes	Joint	Neck
Ears	Hernia	Lung	Other _____		

10. Please circle any of the following for which you have been diagnosed or treated by a physician or health professional:

Alcoholism	Diabetes	Kidney problem
Anemia, sickle cell	Emphysema	Mental illness
Anemia, other	Epilepsy	Neck strain
Asthma	Eye problems	Obesity
Back strain	Gout	Osteoporosis
Bleeding trait	Hearing loss	Phlebitis
Bronchitis, chronic	Heart problems	Rheumatoid arthritis
Cancer	High blood pressure	Stroke
Cirrhosis, liver	Hypoglycemia	Thyroid problem
Concussion	Hyperlipidemia	Ulcer
Congenital defect	Infectious mononucleosis	Other _____

11. Circle all medicine taken in last 6 months:

Blood thinner	Epilepsy medication	Nitroglycerin
Diabetic pill	Heart-rhythm medication	Estrogen
Digitalis	High-blood-pressure medication	Thyroid
Diuretic	Insulin	Corticosteroids
Asthma	Other _____	

12. Any of these health symptoms that occurs frequently is the basis for medical attention. Circle the number indicating how often you have each of the following:

1 = Practically never 2 = Infrequently 3 = Sometimes 4 = Fairly often 5 = Very often

a. Cough up blood
1 2 3 4 5

b. Abdominal pain
1 2 3 4 5

c. Low back pain
1 2 3 4 5

d. Leg pain
1 2 3 4 5

e. Arm or shoulder pain
1 2 3 4 5

f. Chest pain
1 2 3 4 5

g. Swollen joints
1 2 3 4 5

h. Feel faint
1 2 3 4 5

I. Dizziness
1 2 3 4 5

j. Breathless with slight exertion
1 2 3 4 5

Part 3. Health-related behavior

13. Do you now smoke? Yes No

14. If you are a smoker, indicate number smoked per day:

Cigarettes:	40 or more	20-39	10-19	1-9
Cigars or pipes only:	5 or more or any inhaled		Less than 5, none inhaled	

15. Weight now: _____lb. One year ago: _____lb.. Age 21: _____lb.

16. Thinking about the things you do at work, how would you rate yourself as to the amount of physical activity you get compared with others of your age and sex?

1. Much more active
2. Somewhat more active
3. About the same
4. Somewhat less active
5. Much less active
6. Not applicable

17. Now, thinking about the things you do outside of work, how would you rate yourself as to the amount of physical activity you get compared with others of your age and sex?

1. Much more active
2. Somewhat more active
3. About the same
4. Somewhat less active
5. Much less active
6. Not applicable

18. Do you regularly engage in strenuous exercise or hard physical labor?

1. Yes (answer question # 19) 2. No (stop)

19. Do you exercise or labor at least three times a week?

1. Yes 2. No

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE (October 2002)

LONG LAST 7 DAYS SELF-ADMINISTERED FORMAT

FOR USE WITH YOUNG AND MIDDLE-AGED ADULTS (15-69 years)

The International Physical Activity Questionnaires (IPAQ) comprises a set of 4 questionnaires. Long (5 activity domains asked independently) and short (4 generic items) versions for use by either telephone or self-administered methods are available. The purpose of the questionnaires is to provide common instruments that can be used to obtain internationally comparable data on health–related physical activity.

Background on IPAQ

The development of an international measure for physical activity commenced in Geneva in 1998 and was followed by extensive reliability and validity testing undertaken across 12 countries (14 sites) during 2000. The final results suggest that these measures have acceptable measurement properties for use in many settings and in different languages, and are suitable for national population-based prevalence studies of participation in physical activity.

Using IPAQ

Use of the IPAQ instruments for monitoring and research purposes is encouraged. It is recommended that no changes be made to the order or wording of the questions as this will affect the psychometric properties of the instruments.

Translation from English and Cultural Adaptation

Translation from English is encouraged to facilitate worldwide use of IPAQ. Information on the availability of IPAQ in different languages can be obtained at www.ipaq.ki.se. If a new translation is undertaken we highly recommend using the prescribed back translation methods available on the IPAQ website. If possible please consider making your translated version of IPAQ available to others by contributing it to the IPAQ website. Further details on translation and cultural adaptation can be downloaded from the website.

Further Developments of IPAQ

International collaboration on IPAQ is on-going and an ***International Physical Activity Prevalence Study*** is in progress. For further information see the IPAQ website.

More Information

More detailed information on the IPAQ process and the research methods used in the development of IPAQ instruments is available at www.ipaq.ki.se and Booth, M.L. (2000). *Assessment of Physical Activity: An International Perspective.* Research Quarterly for Exercise and Sport, 71 (2): s114-20. Other scientific publications and presentations on the use of IPAQ are summarized on the website.

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** and **moderate** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

PART 1: JOB-RELATED PHYSICAL ACTIVITY

The first section is about your work. This includes paid jobs, farming, volunteer work, course work, and any other unpaid work that you did outside your home. Do not include unpaid work you might do around your home, like housework, yard work, general maintenance, and caring for your family. These are asked in Part 3.

1. Do you currently have a job or do any unpaid work outside your home?

   ☐ Yes

   ☐ No → ***Skip to PART 2: TRANSPORTATION***

The next questions are about all the physical activity you did in the **last 7 days** as part of your paid or unpaid work. This does not include traveling to and from work.

2. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, heavy construction, or climbing up stairs **as part of your work**? Think about only those physical activities that you did for at least 10 minutes at a time.

   _____ **days per week**

   ☐ No vigorous job-related physical activity → ***Skip to question 4***

3. How much time did you usually spend on one of those days doing **vigorous** physical activities as part of your work?

   _____ **hours per day**
   _____ **minutes per day**

4. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads **as part of your work**? Please do not include walking.

   _____ **days per week**

   ☐ No moderate job-related physical activity → ***Skip to question 6***

5. How much time did you usually spend on one of those days doing **moderate** physical activities as part of your work?

_____ **hours per day**
_____ **minutes per day**

6. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **as part of your work**? Please do not count any walking you did to travel to or from work.

_____ **days per week**

☐ No job-related walking → ***Skip to PART 2: TRANSPORTATION***

7. How much time did you usually spend on one of those days **walking** as part of your work?

_____ **hours per day**
_____ **minutes per day**

PART 2: TRANSPORTATION PHYSICAL ACTIVITY

These questions are about how you traveled from place to place, including to places like work, stores, movies, and so on.

8. During the **last 7 days**, on how many days did you **travel in a motor vehicle** like a train, bus, car, or tram?

_____ **days per week**

☐ No traveling in a motor vehicle → ***Skip to question 10***

9. How much time did you usually spend on one of those days **traveling** in a train, bus, car, tram, or other kind of motor vehicle?

_____ **hours per day**
_____ **minutes per day**

Now think only about the **bicycling** and **walking** you might have done to travel to and from work, to do errands, or to go from place to place.

10. During the **last 7 days**, on how many days did you **bicycle** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐ No bicycling from place to place → ***Skip to question 12***

11. How much time did you usually spend on one of those days to **bicycle** from place to place?

_____ **hours per day**
_____ **minutes per day**

12. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐ No walking from place to place → ***Skip to PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY***

13. How much time did you usually spend on one of those days **walking** from place to place?

_____ **hours per day**
_____ **minutes per day**

PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY

This section is about some of the physical activities you might have done in the **last 7 days** in and around your home, like housework, gardening, yard work, general maintenance work, and caring for your family.

14. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, chopping wood, shoveling snow, or digging **in the garden or yard**?

_____ **days per week**

☐ No vigorous activity in garden or yard → ***Skip to question 16***

15. How much time did you usually spend on one of those days doing **vigorous** physical activities in the garden or yard?

_____ **hours per day**
_____ **minutes per day**

16. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, sweeping, washing windows, and raking **in the garden or yard**?

_____ **days per week**

☐ No moderate activity in garden or yard → ***Skip to question 18***

17. How much time did you usually spend on one of those days doing **moderate** physical activities in the garden or yard?

_____ **hours per day**
_____ **minutes per day**

18. Once again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, washing windows, scrubbing floors and sweeping **inside your home**?

_____ **days per week**

☐ No moderate activity inside home → ***Skip to PART 4: RECREATION, SPORT AND LEISURE-TIME PHYSICAL ACTIVITY***

19. How much time did you usually spend on one of those days doing **moderate** physical activities inside your home?

_____ **hours per day**
_____ **minutes per day**

PART 4: RECREATION, SPORT, AND LEISURE-TIME PHYSICAL ACTIVITY

This section is about all the physical activities that you did in the **last 7 days** solely for recreation, sport, exercise or leisure. Please do not include any activities you have already mentioned.

20. Not counting any walking you have already mentioned, during the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **in your leisure time**?

_____ **days per week**

☐ No walking in leisure time → ***Skip to question 22***

21. How much time did you usually spend on one of those days **walking** in your leisure time?

_____ **hours per day**
_____ **minutes per day**

22. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like aerobics, running, fast bicycling, or fast swimming **in your leisure time**?

_____ **days per week**

☐ No vigorous activity in leisure time → ***Skip to question 24***

23. How much time did you usually spend on one of those days doing **vigorous** physical activities in your leisure time?

_____ **hours per day**
_____ **minutes per day**

24. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like bicycling at a regular pace, swimming at a regular pace, and doubles tennis **in your leisure time**?

_____ **days per week**

☐ No moderate activity in leisure time → ***Skip to PART 5: TIME SPENT SITTING***

25. How much time did you usually spend on one of those days doing **moderate** physical activities in your leisure time?
_____ **hours per day**
_____ **minutes per day**

PART 5: TIME SPENT SITTING

The last questions are about the time you spend sitting while at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television. Do not include any time spent sitting in a motor vehicle that you have already told me about.

26. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekday**?

_____ **hours per day**
_____ **minutes per day**

27. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekend day**?

_____ **hours per day**
_____ **minutes per day**

This is the end of the questionnaire, thank you for participating.

	Time Outdoors			Amount of Skin Exposed			
	<5 min	5-30 min	>30min	Hands and face	Hands, face, arms	Hands, face, legs	Bathing suit
Monday	0	1	2	1	2	3	4
Tuesday	0	1	2	1	2	3	4
Wednesday	0	1	2	1	2	3	4
Thursday	0	1	2	1	2	3	4
Friday	0	1	2	1	2	3	4
Saturday	0	1	2	1	2	3	4
Sunday	0	1	2	1	2	3	4

Fig. 1. Scoring for the weekly sun exposure recall questionnaire. Shown in gray are the values ascribed to each category and the method for deriving the Sun Exposure Score (sum of the daily products of Time Outdoors and Skin Exposure). The range of the Sun Exposure Score is from the 0 (lowest amount of time spent outdoors and lowest amount of skin exposed) to a maximum score 56 (outdoors for more than 30 min in a bathing suit every day). (Hanwell et al., 2010)

IRB NUMBER: 1568
IRB EXPIRATION DATE: 11/04/2013

Blood Collection Certificate

This Certificate Verifies that

_____

Participant's ID Number

Is a participant in the **"Vitamin D Status: Associations with Chronic Disease Risk Factors and Cognitive Function"** research study and payment should be charged to account

 # 122727900

This Certificate is valid only with the signature of "investigators name" and a non-duplicate ID Number

Investigator _____

Appendix E

Nutritional Information Sheets

NUTRITIONAL ASSESSMENT
DAILY FOOD DIARY

Name_____

Height_____ Age

Weight_____ Gender F M

Level of Physical Activity Lo Med Hi

FOOD/DRINK CONSUMED	DESCRIPTION	AMOUNT EATEN

IRB APPROVED
IRB NUMBER: 1568
IRB EXPIRATION DATE: 11/04/2013

Calcium/Vitamin D Analysis **Study ID #:** _____

Date:_____ **Circle: Fall 2012 / Spring 2013**

VITAMIN D FFQ/DIET RECALL FORM: Please record how many times you ate/drank this specific food/beverage in the past week and month (4 weeks) and what the typical serving sizes were each time you consumed it? (Please use measuring cup to estimate serving sizes)

Food / Beverage	Frequency/ # of servings in the past week (past 7 days)	Frequency/ # of servings in the past 4 weeks (past 28 days)	Typical serving size(s) each time	Comments
Supplements/multivitamins (Some calcium supps contain vit D)				Type: Brand: Amt. of vit D:
Milk				Type: Don't forget about milk in sauces/casseroles.
Milk beverages (latte, mocha, cappuccino, etc.)				
Soy milk				Brand:
Chocolate milk				
Ice cream				Brand/type:
Whipped cream /Coolwhip				Which one:
Yogurt				Brand:
Cheese (Consider cheese alone & in mixed dishes, such as enchiladas, pizza, casseroles, pasta, etc.)				Note type(s) of cheese:
Butter				
Margarine				
Eggs				Excluding egg whites. Don't forget about omelets, soufflés, frittatas and quiche.
Fish				Don't forget about sushi/sashimi pieces:
Salmon				
Mackerel				
Tuna				
Sardines				
Catfish				
Cod liver oil (NOT including omega 3 supplements)				Note any fish oil sup.
Other				
Mushrooms				Brand:
Liver				
Ready-to-eat cereals				Type/brand:
Ensure or slim fast				Which beverage:
Vit D fortified OJ				
Other vit D fortified food/beverage				

Appendix F

Raw Data

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
SUBJECT	SUB ID	AGE	SEX (1 male)	ANAM AB	HEIGHT	WEIGHT	BMI	WC	PA	GLUCOSE	T CHOL	TRIGLY	HDL	LDL	VIT D	VITD_Status
VITD 01	1	59.9	1	2	184.5	97.5	28.6	44	3	91	236	194	56	141	36.7	3
VITD 02	2	58.3	2	2	162	67.1	25.6	37.5	3	85	191	72	53	124	7.6	1
VITD 03	3	54.3	2	1	167	62.3	22.3	35.5	3	90	177	62	96	69	35.4	3
VITD 05	5	68.9	2	1	151	57.3	25.1	34	2	95	250	126	69	156	25.5	2
VITD 06	6	60.9	2	2	173	77.7	26	38.5	2	87	139	112	38	79	17.8	1
VITD 07	7	63.3	1	2	180	77.1	23.8	37.5	2	86	238	89	73	147	25.3	2
VITD 08	8	62.1	2	2	161	58.6	22.6	32.5	3	81	210	65	113	84	40.8	3
VITD 09	9	61.9	2	1	170.5	112.6	38.7	45	1	139	153	85	47	89	19.3	1
VITD 10	10	62	2	1	160	78.1	30.5	42.5	3	84	212	142	70	114	31.7	3
VITD 11	11	61.8	2	2	162.5	80.7	30.6	41.5	2	87	127	72	45	68	27.1	2
VITD 14	14	66.3	1	2	182	77.5	23.4	36	2	88	258	101	82	156	23.5	2
VITD 15	15	66	2	1	167.5	77.9	27.8	37	1	90	199	112	73	104	10.1	1
VITD 17	17	64.5	2	1	162.5	59.8	22.6	32.5	2	93	215	89	92	105	31.4	3
VITD 18	18	51	2	1	177	96.8	30.9	44.5	2	81	264	184	50	177	34.2	3
VITD 19	19	63.9	2	1	165	66.9	24.6	34	1	84	184	61	98	74	19.1	1
VITD 20	20	57.9	1	1	179	76.6	23.9	37	3	94	184	72	79	91	25.5	2
VITD 23	23	64.1	2	1	162.5	62.3	23.6	31.5	2	101	255	51	100	145	57.7	3
VITD 24	24	53.4	1	1	165.5	64.8	23.7	36	2	94	190	90	64	108	38.3	3
VITD 25	25	68.9	2	2	165	63.4	23.3	33	3	91	173	66	75	85	33.7	3
VITD 26	26	64.7	1	2	181	84.8	25.9	38	3	104	253	88	87	148	30.3	3
VITD 27	27	70.8	1	2	190	91.9	25.5	40.5	3	96	199	85	82	100	26.2	2

R	S	T	U	V	W	X	Y	Z	AA	AB	AC	AD	AE	AF	AG	AH	AI	AJ
% FAT	A/G RATIO	MMSE	SunExSc	TimeSun	SkinExSc	VITD DATE	VITD Syn	amin D (m	VitD IU	VITD Supp	calories (K	otal Fat (g	%Kcal Fat	tary Fiber	bohydrate	%Kcal CHC	Protein (g)	%Kcal PRO
32.6	1.5	29	14	14	7	14-Feb	2	12.8	512	2500	2818	84.8	27.08304	39.2	381.6	54.16608	144.6	20.5252
36.2	1.18	30	22	12	13	14-Feb	2	0.255	10.2	400	2280	94.9	37.46053	18.2	270.6	47.47368	91.9	16.12281
31.6	1.07	30	14	6	10	18-Feb	2	6.084	243.36	1000	3147	122.9	35.14776	58.6	398	50.58786	99.2	12.60883
40	0.98	30	8	6	8	1-Mar	1	0	0	800	2925	145.5	44.76923	23.8	287.4	39.30256	120.2	16.43761
44.1	1.04	30	2	2	7	15-Feb	2	0.06	2.4	0	3133	177.7	51.04692	10.6	257.3	32.8503	133.2	17.00606
25.3	1.24	28	12	6	12	26-Feb	2	0	0	0	1544	53.9	31.41839	17.3	178.4	46.21762	67.2	17.40933
32.9	0.67	29	11	9	8	19-Feb	2	0.98	39.2	2000	1360	46.3	30.63971	17	114.1	33.55882	72	21.17647
50.6	0.94	30	12	12	7	19-Feb	2	0.447	17.88	0	1303	69.1	47.72832	6.4	87.9	26.98388	82.7	25.38757
40.8	1	30	3	3	7	19-Feb	2	0.374	14.96	1200	2637	85.3	29.11263	33.4	344.7	52.28669	126.8	19.23398
49.6	0.99	29	9	9	7	1-Mar	2	0.075	3	400	1935	30.6	14.23256	34.4	317.7	65.67442	113.2	23.40052
21.7	1.39	30	18	12	10	20-Mar	2	4.104	164.16	0	1735	75.9	39.37176	15.9	211.1	48.66859	46	10.60519
46.2	1.02	30	6	6	7	26-Feb	2	0.075	3	0	2063	86.6	37.77993	36.5	226.3	43.87785	57.5	11.14881
38.7	0.71	30	2	1	8	28-Feb	2	0	0	400	1173	33	25.31969	19.4	180.9	61.68798	45.3	15.44757
36.3	1.24	30	14	14	7	5-Mar	1	0.632	25.28	1000	1932	97.4	45.37267	19.1	212.1	43.91304	63.7	13.18841
46.5	0.96	30	7	7	7	13-Mar	1	2.144	85.76	400	1335	45.1	30.40449	13.1	141.2	42.30712	58	17.37828
18.2	1.06	30	13	11	8	14-Mar	1	5.996	239.84	1000	1953	70	32.25806	23.2	230.3	47.16846	111.9	22.91859
39.4	0.91	30	26	11	16	20-Mar	1	1.89	75.6	5000	2746	173.8	56.96286	36.2	163.6	23.83103	146.2	21.29643
28.7	1.09	30	7	7	7	20-Mar	1	7.104	284.16	2200	3081	107.5	31.40214	39.2	388.1	50.38624	149.6	19.42227
37.6	0.83	29	10	6	9	2-Apr	1	6.956	278.24	0	1150	53.8	42.10435	15.8	126.9	44.13913	47.8	16.62609
33.9	1.09	27	36	12	21	9-Apr	1	0	0	500	1804	101.3	50.53769	12.8	167.3	37.09534	58.5	12.97118
33.4	1.13	30	33	10	23	4-Apr	1	2.94	117.6	0	2035	53.9	23.83784	14.4	206.3	40.55037	128.3	25.21867

AK	AL	AM	AN	AO	AP	AQ	AR	AS	AT	AU	AV	AW	AX	AY	AZ	BA
Operator	P_SP	P_DP	P_MEANP	C_AP_HR7	HR	C_AGPH_F	C_SP	C_DP	Throughpı	Throughpı	Throughpı	Throughpı	Throughpı	Throughpı	Throughput_CDD	
98	115	74	86	1	56	4	104	75	245.95	99.04	31.04	35.92	144.05	35.95	15.73	
90	105	62	79	12	62	37	99	63	54.38	79.19	16.84	23.29	121.24	33.18	29.32	
94	120	81	95	6	49	22	115	81	233.58	95.96	15.44	22.44	125.36	38.09	35.13	
91	145	87	112	15	67	32	139	89	211.19	74.53	20.21	25.64	110.39	28.95	26.25	
93	104	72	85	7	87	30	96	74	173.74	72.39	19.65	23.98	132.62	36.1	26.49	
98	122	79	94	6	79	19	111	81	231.93	101.41	13.09	21.52	123.93	32.12	38.23	
100	128	84	102	11	72	30	123	85	225.61	93.58	15.51	26.76	128.21	31.88	16.63	
95	131	77	95	7	70	19	117	78	194.66	87.89	26.19	28.91	105.35	34.58	29.63	
90	123	75	94	14	83	34	114	77	217.45	81.36	28.09	27.97	120.45	40.9	25.25	
100	109	69	85	7	59	23	103	70	197.25	62.79	20	23.04	105.24	25.53	12.54	
100	137	89	109	6	57	15	131	90	215.61	88.58	34.78	24.4	112.16	36.47	30.31	
96	138	88	111	16	70	38	135	90	275.33	119.54	41.04	41.55	143.76	48.18	39.28	
91	106	66	81	7	67	24	98	67	240.68	106.22	20.12	35.48	161.22	44.83	29.95	
87	136	87	103	4	66	13	123	87	234.79	116.03	27.34	37.03	156.33	58.69	42.09	
95	115	76	93	11	59	35	112	77	240.67	107.61	29.09	21.56	133.41	33.27	32.67	
100	142	77	97	2	62	6	119	78	256.19	102.79	27.92	35.28	147.9	46.37	47.31	
100	141	79	100	10	75	22	127	80	252.1	115.26	39.71	40.93	125.6	29.76	57.15	
89	109	72	85	3	56	12	101	73	246.71	85.69	24.51	29.33	116.95	28.69	28.09	
90	104	68	83	12	70	40	100	69	189.8	74.35	16.76	13.77	100.3	28.14	16.6	
92	122	76	86	0	58	0	105	76	237.88	96.82	23.07	23.77	125.01	25.3	20.01	
98	124	72	89	8	75	20	111	73	180.9	71.43	31.52	17.93	73.39	26.21	20.03	

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
VITD 29	29	53.9	2	1	172	70.8	23.9	31	3	90	168	42	69	91	48.6	3
VITD 31	31	59	1	2	181	123.6	37.7	50	2	128	157	120	52	81	29	2
VITD 32	32	60.9	2	1	161.5	75.4	28.9	38	3	92	227	115	60	144	34.1	3
VITD 33	33	50.9	2	1	155	62.8	26.1	37	3	90	158	219	63	51	21.5	2
VITD 34	34	67	1	1	184.5	98.7	29	41	2	90	147	120	45	78	31.3	3
VITD 35	35	69	2	2	165	55.7	20.5	32	2	82	224	68	77	133	53.1	3
VITD 36	36	63.8	2	1	164	80.7	30	42	2	83	267	132	85	156	33.8	3
VITD 37	37	65.6	1	1	174	78.9	26.1	37	3	87	189	104	51	117	32.4	3
VITD 38	38	62.5	1	2	191	92.4	25.3	40	3	88	166	62	57	97	38.9	3
VITD 39	39	55.9	2	1	152.5	94.7	40.7	42	2	82	181	162	60	89	29.3	2
VITD 40	40	56.9	2	1	159	126.9	50.2	57	1	91	194	56	63	120	31.9	3
VITD 41	41	56	2	1	164	77.3	28.7	37	2	83	177	143	58	90	27.3	2
VITD 42	42	53.2	2	1	154	52.8	22.3	30	3	80	165	58	78	75	37.7	3
VITD 43	43	65.5	2	2	156	88.4	36.3	47	2	89	206	272	48	104	50.8	3
VITD 44	44	51.8	2	1	155	59	24.6	32	2	87	181	77	68	98	34.4	3
VITD 45	45	51	2	1	165	60.6	22.3	31	3	84	171	80	74	81	21.6	2
VITD 48	48	67.9	2	1	178	66.4	21	29	3	94	207	53	98	98	48.3	3
VITD 49	49	63.8	2	2	160	55.5	21.6	28.5	3	75	214	63	63	138	39.6	3
VITD 51	51	62.7	2	1	164	68.1	25.3	39	2	83	215	122	67	124	35.9	3
VITD 52	52	58.4	1	1	171.5	82.8	28.2	38	3	95	259	129	55	178	20.6	2
VITD 54	54	62.5	2	2	166	84.4	30.6	42	1	114	174	119	49	101	20.8	2
VITD 55	55	61.6	2	1	157.5	53.7	21.6	32.5	1	78	192	94	91	82	26.2	2

R	S	T	U	V	W	X	Y	Z	AA	AB	AC	AD	AE	AF	AG	AH	AI	AJ
33.9	0.64	30	22	9	16	15-Apr	1	15.4	616	1500	2364	60.2	22.91878	36.9	334.8	56.64975	95.1	16.09137
39.3	1.19	30	9	7	9	22-Apr	1	0	0	400	1237	32.6	23.71867	43.3	166.3	53.77526	92.1	29.78173
39.2	1.13	28	14	14	7	11-Apr	1	14.4	576	2400	3628	143.7	35.64774	48.4	503	55.45755	127.2	14.02426
42.8	1.09	30	6	2	11	22-May	1	0.96	38.4	0	1765	73.1	37.27479	18	223.8	50.71955	65	14.73088
31.1	1.05	30	33	11	21	29-May	1	3.927	157.08	1000	2018	75.2	33.53816	12.8	257.2	50.98117	81.9	16.23389
26.5	0.65	29	30	14	15	29-May	1	2.062	82.48	2000	1759	71.4	36.53212	20.6	225.7	51.32462	67	15.23593
43.3	0.98	30	24	12	14	31-May	1	0.192	7.68	800	1568	66	37.88265	25	187.2	47.7551	71.6	18.26531
29.6	1.26	30	22	9	16	18-Jun	1	0	0	1800	1521	56	33.13609	11.9	141.6	37.23866	89	23.40565
31.4	1.09	30	42	14	21	24-Jul	1	0	0	800	1855	46.2	22.41509	26.2	250.6	54.03774	113.9	24.56065
55.2	1.05	29	20	8	13	24-Jun	1	0	0	1000	1424	36.5	23.06882	31.1	231.8	65.11236	53.4	15
56.5	0.99	29	6	3	9	28-Jun	1	1.276	51.04	10000	1474	67.3	41.09227	25	145.8	39.56581	75.1	20.37992
46.5	1	30	27	9	21	27-Jun	1	0.585	23.4	0	1344	49	32.8125	8.389	189.8	56.4881	43.7	13.00595
30	0.78	30	46	14	23	28-Jun	1	0.2	8	400	2557	97.3	34.24716	21.6	328.1	51.32577	73.9	11.56042
55.5	1.1	30	8	4	19	15-Jul	1	0.093	3.72	6500	1469	78.7	48.21647	12.4	176.4	48.03268	22.4	6.099387
34.1	0.86	30	29	10	19	10-Jul	1	2.488	99.52	700	2183	115.4	47.57673	29.9	181.6	33.27531	121.4	22.24462
35.7	0.87	30	22	9	16	12-Jul	1	0	0	0	1115	18.5	14.93274	20.3	206.4	74.04484	34.8	12.4843
28.5	0.69	29	42	14	21	17-Jul	1	0.426	17.04	3500	1398	52	33.47639	9.849	149.6	42.80401	52.5	15.02146
32.4	0.82	29	24	8	15	17-Jul	1	3.47	138.8	0	1273	35.8	25.31029	22	154.8	48.64101	91	28.59387
46.4	1.05	29	38	14	19	17-Jul	1	1.338	53.52	2500	1461	63.4	39.05544	33.5	153.1	41.9165	78.4	21.46475
25.5	1.43	30	17	6	17	17-Jul	1	2.464	98.56	0	2008	87.5	39.21813	16.6	214.4	42.70916	92.1	18.34661
47.2	0.97	30	18	11	12	29-Jul	1	0.159	6.36	400	1205	46.7	34.87967	10.6	150.1	49.82573	48.5	16.09959
34.1	0.73	30	10	5	14	12-Aug	1	0.65	26	0	990.2	22.9	20.81398	11.5	133.8	54.04969	29.8	12.03797

AK	AL	AM	AN	AO	AP	AQ	AR	AS	AT	AU	AV	AW	AX	AY	AZ
98	119	73	86	-1	49	-3	104	73	213.37	103.86	21.98	38.73	132.66	41.91	42.29
97	144	79	95	-2	45	-5	122	79	209.47	88.4	17.6	23.09	115.76	37.65	43.8
96	132	81	101	10	69	26	122	82	213.69	93.53	30.48	25.83	117.46	39.06	36.66
97	108	78	90	4	50	20	104	79	222.79	109.3	31.81	40.77	138.6	48.55	45.87
99	123	74	88	2	69	6	107	74	248.27	86.14	26.14	22.28	122.55	28.96	27.96
100	121	72	93	14	65	35	118	73	165.06	95.27	24.36	16.93	127.47	19.13	27.41
91	123	76	95	10	56	27	118	77	209.97	98.37	24.71	37.95	132.29	45.12	35.23
94	125	83	97	3	75	10	114	83	229.31	95.24	26.77	31.25	159.84	47.69	52.36
100	118	77	93	9	60	28	113	78	262.01	94.4	20.18	20.87	111.43	37.41	31.8
97	104	72	84	5	62	22	98	72	208.4	106.05	23.42	28.65	121.07	46.62	28.57
100	113	79	92	6	73	23	105	80	227.34	112.99	33.43	29.75	156.29	29.68	35.94
98	106	76	86	4	72	19	98	77	242.13	109.13	13.5	36.54	122.53	36.82	50.4
99	131	89	106	11	48	37	129	90	211.57	97.82	24.22	24.19	104.41	45.93	47.7
100	108	68	83	9	73	29	101	69	161.41	80.01	24.09	27.47	106.06	42.4	18.9
85	131	82	103	11	60	28	127	84	257.4	100.62	21.27	29.43	134.63	38.36	24.2
85	102	73	84	5	65	22	96	74	214.59	106.45	25.94	33.23	138.67	43.48	47.01
96	130	76	95	14	44	35	127	76	227.98	94.27	31.93	25.01	121.35	31.24	34.16
99	104	67	82	12	69	40	99	68	188.89	79.39	20.63	13.35	111.96	34.53	39.76
82	157	91	116	17	65	33	149	92	187.05	111.36	30.84	36.45	128.51	39.85	48.53
100	141	92	109	6	61	18	132	93	218.26	102.07	27.8	35.72	129.21	37.81	44.14
94	108	83	93	7	83	35	103	84	159.82	93.57	13.54	24.37	121.81	33.75	22.87
100	112	71	86	9	84	29	102	72	167.26	97.51	27.6	36.24	142.46	35.13	45.34

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
VITD 56	56	58.4	2	1	166	67.5	24.5	35	2	87	193	73	79	99	37	3
VITD 57	57	60.9	2	1	162.5	64.9	24.6	35.5	3	94	318	160	54	232	24.1	2
VITD 58	58	56.3	2	1	154	57.8	24.4	33	2	94	170	135	59	84	41.5	3
VITD 59	59	53.2	2	2	173	87.1	29.1	42	2	88	179	131	55	98	23.5	2
VITD 60	60	50.6	2	2	167.5	87.3	31.1	43.5	2	95	168	119	48	96	51	3
VITD 61	61	62.3	1	2	173.5	69.5	23.1	33.5	2	87	206	58	70	124	22.6	2
VITD 63	63	60.9	2	1	160	55.7	21.8	30	3	90	171	104	40	110	46.3	3
VITD 64	64	61.5	1	1	181	79	24.1	36.5	2	92	169	149	39	100	27.5	2
VITD 67	67	60.1	2	1	159.5	86.4	34	42	1	103	231	252	37	144	14.2	1
VITD 68	68	55.8	2	1	168.5	75.9	26.7	41	3	95	217	104	82	114	14.4	1
VITD 69	69	56.6	2	2	172	99.7	33.7	42	2	96	227	163	62	132	31.4	3
VITD 70	70	60.3	2	1	160	63.1	24.6	33.5	3	152	236	122	79	133	16.3	1
VITD 71	71	59.8	2	2	165	67.3	24.7	39	2	87	168	131	54	88	30.9	3
VITD 73	73	58.9	2	1	160	86.2	33.7	40.5	2	99	179	113	49	107	25.1	2
VITD 74	74	53.6	2	1	174	65.6	21.7	33	2	83	193	64	60	120	25.3	2
VITD 75	75	62.5	2	1	158	90.3	36.2	46	2	85	175	77	58	102	20.5	2
VITD 76	76	54.6	2	2	159	79.7	31.5	41	2	81	203	120	48	131	56.5	3
VITD 77	77	70.6	2	2	161.5	51.7	19.8	31.5	3	92	217	107	69	127	29	2
VITD 78	78	58.6	2	1	146	49.5	23.2	33.5	1	86	159	43	74	76	36.4	3
VITD 79	79	69.3	1	1	172	75.2	25.4	38	2	90	188	74	65	108	29.2	2
VITD 80	80	62.5	1	1	165	81.8	30	40.5	2	107	186	136	43	116	20.4	2
VITD 81	81	52.6	2	2	161.5	75.8	29.1	39	3	92	182	59	62	108	36.5	3

R	S	T	U	V	W	X	Y	Z	AA	AB	AC	AD	AE	AF	AG	AH	AI	AJ
43.6	0.9	30	15	9	11	30-Jul	1	8.031	321.24	1000	1736	45.9	23.79608	27.8	242.1	55.78341	101.7	23.43318
34.3	1.1	30	25	11	16	2-Aug	1	1.585	63.4	0	1659	53.7	29.13201	18.7	172.2	41.51899	71	17.11875
37.6	0.86	30	44	14	22	6-Aug	1	6.454	258.16	1000	1842	67.5	32.98046	13.7	188.9	41.02063	100.5	21.8241
48.9	1.02	30	24	9	17	7-Aug	1	0	0	0	1029	40.4	35.33528	7.713	115.1	44.74247	52.1	20.25267
49.8	1.09	28	16	8	14	7-Aug	1	1.65	66	5000	1513	47	27.9577	23.8	200.3	52.9544	81.7	21.59947
17.2	1.02	30	15	9	10	24-Oct	1	0.975	39	400	2847	104.7	33.098	29.1	380.2	53.41763	95.2	13.37548
32.1	0.76	30	46	14	23	19-Aug	1	0	0	0	1290	33.9	23.65116	7.823	121	37.51938	83	25.73643
31	1.15	30	16	8	14	16-Aug	1	4.101	164.04	0	2557	71.3	25.09582	30.6	407.6	63.76222	88.4	13.82871
51.9	0.98	30	14	10	9	23-Aug	1	0.168	6.72	0	970.5	33.3	30.88099	7.871	130.5	53.78671	41	16.89851
43.4	1.26	30	12	12	7	16-Aug	1	0.09	3.6	0	1316	52.7	36.04103	11.9	124.9	37.96353	90.1	27.38602
48.8	0.93	30	36	14	18	16-Aug	1	0.085	3.4	0	1296	45.2	31.38889	13.7	121.7	37.56173	81.6	25.18519
40.8	1.04	30	27	9	21	20-Aug	1	0.367	14.68	0	1832	85.4	41.95415	24.4	176.2	38.47162	47.5	10.37118
43.1	0.91	29	27	9	19	26-Aug	1	0	0	0	1841	78.3	38.27811	14.2	232.3	50.47257	63	13.68821
52.7	0.95	29	27	9	21	28-Aug	1	1.429	57.16	1000	2995	168.8	50.72454	21.2	200.8	26.81803	134.8	18.00334
25.5	0.85	30	27	9	19	10-Sep	1	7.044	281.76	500	2860	119.1	37.47902	27.1	337.2	47.16084	120.1	16.7972
45.1	1.16	30	44	14	22	5-Sep	1	3.086	123.44	0	1052	37.5	32.08175	21.7	134.4	51.10266	54.4	20.68441
47.5	0.97	30	3	1	9	10-Sep	1	0.17	6.8	5000	1977	68.5	31.18361	23.6	271.6	54.95195	78.8	15.94335
34.7	0.91	28	29	13	15	19-Sep	1	0.962	38.48	400	1367	70	46.08632	21.6	149.7	43.80395	53.2	15.56693
35.9	0.99	30	0	0	7	17-Sep	1	0.255	10.2	1200	1194	38.6	29.09548	19.2	151.5	50.75377	71.8	24.0536
34.6	1.19	30	15	5	17	19-Sep	1	1.132	45.28	1000	1424	65.8	41.58708	28.1	175.3	49.24157	46.8	13.14607
30.1	1.32	30	16	8	13	19-Sep	1	2.94	117.6	0	667.6	19.1	25.74895	10.1	103.5	62.01318	27.5	16.47693
42.2	0.85	30	28	14	14	7-Oct	1	0.227	9.08	1200	1968	74.3	33.97866	30.4	257.4	52.31707	84.5	17.1748

AK	AL	AM	AN	AO	AP	AQ	AR	AS	AT	AU	AV	AW	AX	AY	AZ
85	110	76	91	11	84	39	104	78	230.63	106.96	24.87	20.71	128.55	39.57	26.45
100	136	85	105	11	78	27	126	87	238.17	115.95	28.22	33	149.37	51.65	60.72
99	127	76	96	9	53	23	121	77	250.34	112.6	32.83	22.99	138.24	49.36	44.9
96	117	78	92	6	72	21	108	79	168.57	73.25	26.9	27.54	106.65	41.7	17.8
88	122	80	96	12	71	36	116	81	230.39	98.76	20.91	8.95	130.55	31.84	41.14
93	131	80	99	8	67	22	120	81	254.26	109.63	26.44	23.79	133.95	31.66	29.65
99	116	73	90	7	61	23	109	74	240.12	107.04	30.99	33.45	133.37	38.71	39.86
97	124	73	90	3	70	10	110	74	242.6	97.97	23.66	30.4	123.15	29.38	45.4
99	139	82	101	9	66	21	128	83	198.31	108.96	32.03	23.14	115.6	45.88	37.79
66	119	74	94	14	65	38	116	76	202.69	82.23	24.82	30.41	116.91	37.42	30.85
99	122	86	100	8	84	27	114	88	192.59	79.23	29.23	33.05	121.91	37.7	30.7
84	136	84	105	12	61	29	130	85	189.45	124.39	28.76	37.41	149.94	37.83	28.86
100	127	75	94	16	54	44	120	76	201.19	85.36	25.26	16.27	2.91	30.77	26.05
95	116	73	91	12	72	34	111	74	214.69	107.4	32.81	37.1	130.99	38.75	27.02
99	110	70	86	7	65	24	103	71	221.19	84.1	29.53	33.74	124.58	47.99	42.88
100	162	105	130	17	53	40	159	106	191.57	95.15	20.69	27.65	136.2	34.68	30.06
100	114	75	91	7	64	25	107	76	222.56	109.52	37.24	38.87	129.98	44.81	54.85
99	114	68	85	10	59	27	108	69	225.16	97.86	25.39	28.91	114.63	28.69	18.56
92	120	74	91	12	63	34	114	75	213.05	84.02	26.3	16.66	118.49	42.68	23.85
100	110	72	86	4	57	14	101	73	192.4	89.56	18.63	33.6	118.41	36.86	46.19
94	127	76	93	4	66	13	113	77	209.33	101.84	22.94	20.62	126.73	44.33	40.94
93	119	72	90	5	55	16	110	73	199.06	69.73	19.73	22.34	134.94	36.1	37.38

VITD 82	82	58.9	1	1	171	79.4	27.2	38.5	2	130	132	167	50	49	28.3	2
VITD 83	83	51.8	2	1	164	86	32	43	1	86	197	110	55	120	33.9	3
VITD 84	84	59.3	2	2	160.5	49.9	19.4	34	3	87	173	84	72	84	53.1	3
VITD 85	85	64.9	1	1	177	87.6	28	38	3	109	183	67	67	103	24	2
VITD 86	86	62.3	2	2	150	60.1	26.7	33	3	85	163	96	70	74	51.3	3
VITD 87	87	50	2	2	157	65.1	26.4	41	1	307	248	835	29		17.9	1
VITD 88	88	54	2	1	162.5	83.4	31.6	43	3	92	197	102	64	113	27.6	2

32.5	1.29	30	18	9	14	7-Oct	1	2.188	87.52	0	2483	88.8	32.18687	30.2	301.6	48.58639	137.2	22.1023
50.4	0.97	30	14	8	12	7-Oct	1	1.196	47.84	400	1232	56.2	41.05519	15.2	134.1	43.53896	56.1	18.21429
29.7	0.82	30	3	3	7	9-Oct	1	0.03	1.2	1000	1791	74.8	37.58794	23.8	235.8	52.66332	66.3	14.80737
27	1.17	30	14	14	7	1-Nov	2	6.134	245.36	600	2807	130.9	41.97007	11.9	299	42.60777	93.6	13.33808
41.2	0.93	30	26	13	14	13-Nov	2	1.719	68.76	1000	1376	51.2	33.48837	16.7	150.7	43.80814	82.6	24.01163
42.5	1.2	29	15	8	12	13-Nov	2	0.196	7.84	400	1131	48.1	38.27586	13.4	139.6	49.37224	41.2	14.57118
45.8	1.03	30	10	5	11	15-Nov	2	4.958	198.32	2000	2239	84.6	34.00625	15.6	304.3	54.36356	75	13.39884

95	134	82	99	8	63	22	123	83	220.08	92.97	15.28	50.16	119.5	52.8	53.72
100	126	87	103	12	69	39	121	88	234.56	98.96	34.43	35.78	139.79	39.71	27.18
98	140	84	108	15	66	32	135	86	210.67	83.59	20.5	17.17	107.34	25.39	32.47
98	130	85	99	2	66	8	116	86	268.88	101.11	30.39	40.37	129.16	36.83	37.2
91	104	69	83	10	76	35	98	70	207.49	96.75	9.17	28.99	117.56	31.13	14.82
99	107	76	88	5	70	23	100	77	184.52	79.58	13.51	14.31	112	25.32	23.97
97	130	88	105	14	77	39	125	89	246.27	110.75	32.63	28.84	150.65	34.84	41.52